ASSOCIATION OF MATRIX METALLOPROTEINASE-2 GENE PROMOTER POLYMORPHISM AND THE ASSOCIATED PHENOTYPE VARIATION WITH MYOCARDIAL INFARCTION

Dissertation submitted for

M.D. BIOCHEMISTRY BRANCH – XIII
DEGREE EXAMINATION

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI – 600 032
TAMIL NADU

APRIL 2013

BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled **ASSOCIATION OF MATRIX METALLOPROTEINASE-2 GENE PROMOTER POLYMORPHISM AND THE ASSOCIATED PHENOTYPIC VARIATION WITH MYOCARDIAL INFARCTION** is the original bonafide work done by **Dr.B.SudhaPresanna**, Post Graduate Student, Institute of Biochemistry, Madras Medical College, Rajiv Gandhi Gandhi General Hospital, Chennai, under our direct supervision and guidance.

Prof.Dr.Pragna B.Dolia.MD.,
Former Director and
Professor (Guide)
Institute of Biochemistry
Madras Medical College
Chennai – 600 003.

Prof.Dr.M.Shyamraj.MD.,
Director and Professor
Institute of Biochemistry
Madras Medical College
Chennai – 600 003.

Dean
Rajiv Gandhi Government General Hospital
Madras Medical College,
Chennai – 600 003.

SPECIAL ACKNOWLEDGEMENT

The author gratefully acknowledges and sincerely thanks Professor **Dr. V.Kanagasabai M.D,** Dean, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai, for granting his permission to utilize the facilities of this Institution for the study.

ACKNOWLEDGEMENT

The author expresses her warmest respects and profound gratitude to **Dr.M.Shyamraj, M.D.,** Director and Professor, Institute of Biochemistry, Madras Medical College, Chennai, for his able guidance, constant encouragement , support and valuable time but for which this dissertation could not have been made possible.

With extreme gratitude, the author acknowledges **Dr. Pragna B.Dolia, M.D,** former Director and Professor, my guide ,for her constant guidance and keen interest and encouragement during the course of the study.

The author is extremely thankful to **Dr.V.E.Dhandapani , M.D., DM** (cardiology)., Professor and Head of the Department, Institute of Cardiology, Rajiv Gandhi Government General Hospital, Chennai, for granting permission to obtain blood samples from the patients.

The author expresses her sincere gratitude to Associate Professors **Dr. K.Ramadevi M.D, Dr.R.Chitraa M.D, Dr.V.Amudhavalli M.D, Dr. I.Periyandavar M.D, Dr.V.K.Ramadesikan M.D, Dr.S.Sumathi M.D,** Institute of Biochemistry, Madras Medical College, for their guidance and support.

The author expresses her warm respects and sincere thanks to Assistant Professors **Dr.C.Shanmugapriya, Dr.PoonguzhaliGopinath, Dr.V.Ananthan, Dr.V.G.Karpagavalli, Dr. C.Mythili, Dr.S.Siva,** Assistant

Professors, Institute of Biochemistry, Madras Medical college for their guidance and constant encouragement.

The author expresses her special thanks to her colleagues for their immense help, constant encouragement and unconditional support through out the study.

The author gratefully acknowledges the help rendered by **Mr.Boopathy Kangusamy,** Statistician, during the statistical analysis of the study.

The author is indebted to the patients and the persons from whom blood samples were collected for conducting the study.

Finally , the author expresses her special thanks to her family members for the moral support and encouragement extended by them.

INDEX

ABBREVIATION

CHD	–	Coronary Heart Disease
VLDL	–	Very Low Density Lipoprotein
LDL	–	Low Density Lipoprotein
HDL	–	High Density Lipoprotein
ICAM1	–	Intercellular Cell Adhesion Molecule 1
VCAM1	–	Vascular Cell Adhesion Molecule 1
PeCAM1	–	Pericellular Cell Adhesion Molecule1
IL1	–	Interleukin 1
TNF-α	–	Tumour Necrosis Factor-α
MMP-2	–	MatrixMetalloProteinase-2
ECM	–	Extra Cellular Matrix
ROS	–	Reactive Oxygen Species
eNOS	–	Endothelial Nitric Oxide Synthase
TIMP	–	Tissue Inhibitor MetalloProteinase
MT-MMP	–	MembraneTy pe MetalloProteinase

SMK	–	Smoking
ALC	–	Alcoholism
BMI	–	Body Mass Index
DM	–	Diabetes Mellitus
HYT	–	HyperTension
MI	-	Myocardial Infarction
WT	–	Weight
HT	–	Height
CHOL	–	Cholesterol
TGL	–	Triglyceride
EDTA	–	Ethylene Diamine Tetra Acetic Acid
DNA	–	Deoxyribonucleic acid
ELISA	-	Enzyme Linked Immunosorbent Asssy
HRP	-	Horse Radish Peroxidase
TMB	-	Tetra Methyl Benzidine

Introduction

INTRODUCTION

In many developing countries Myocardial infarction has becoming a major problem in public health [1,2]. MI is a multifactorial disease caused by genetic and environmental factors.The major cause of death in the world is Myocardial infarction [3]. The high plasma lipid levels, high plasma glucose levels,high blood pressure, obesity, smoking, and family history of cardiac disease are the most important risk factors for MI. MI is mainly due to atherosclerosis of the coronary arteries . The structural changes, which permits the accumulation of cells, extracellular matrix and lipids in the intimate layer of the diseased artery and allows the growth of atherosclerotic plaque. The fibrous cap lining atheromatous plaque gets ruptured, gives rise to thrombosis, and its complications[4].

Pathophysiology of MI involves a wide variety of proteins, including the matrix metalloproteinases (MMPs).Atheromatous plaque formation is facilitated by the action of MMPs. Major extra cellular components of the basal lamina around blood vessels such as type 1V collagen, laminin, and fibronectin are degraded by MMP-2. MMPs also weakens the arterial wall, resulting destabilizing of atheromatous plaque and dissolution of fibrous cap leading to MI[5].

Matrix metalloproteinases are zinc dependent endopeptidases that degrade components of the extracellular matrix (ECM). 72KDa type

IV collagenase also known as MMP-2, is an ubiquitous metalloproteinase involved in various functions such as vascular remodeling, atheromatous plaque rupture, and degrading matrix proteins. MMP-2, also known as gelatinase A . In human, it is encoded by the MMP-2 gene[6].

MMP-2 is produced as zymogen, after the production pro MMP-2 is thought to be bound to its specific inhibitor, called as tissue inhibitor of matrix metalloproteinases-2 . There are several pathways for activation of the proenzyme, but mainly the most important pathway is activation by membrane type metalloproteinases-1 (MT1-MMP)[7]. MTI-MMP binds to TIMP-2,this complexed structure comes near to the active site of the MT-MMP enzyme. This results to removal of two specific pro peptides from pro MMP-2 and the production of an active 72 KDa MMP-2 enzyme[8].

The gene for human MMP-2 contains 27,862 basepair genomic DNA and is composed of 13 exons. It has been localized on chromosome 16q21[9]. Several common restriction fragment length polymorphisms (RFLPs) have been reported in the MMP-2 gene locus. MMP-2 gene –1306C>T promoter region is linked with development of MI[10]. This base transition is situated in CCACC box of the sp1 binding site.

Increased MMP-2 levels have been found in the plasma of patients with MI[11]. Elevated MMP-2 has also been found in atherosclerotic plaques of coronary arteries[12].

In view of this we have evaluated the distribution of MMP-2 promoter gene polymorphism by PCR- RFLP and the concerned phenotype (MMP-2) was analyzed by using ELISA.

Review of Literature

REVIEW OF LITERATURE

Coronary heart disease has been known as impairment of function of heart due to insufficient blood supply to the heart. Atherosclerosis is a multifactorial disease. The risk of coronary artery disease is associated with an individual's genetic and environmental factors.[13]. A large number of studies such as the Framingham heart study[14], the Helsenki Heart study, have been conducted to examine the role of risk factors for coronary artery disease. The risk factors identified by these epidemiological studies include a group of modifiable risk factors like blood lipid profile abnormalities, hypertension, physical inactivity, obesity, cigarette smoking, alcoholism, diabetes mellitus, hyper homocysteinemia. Though overwhelming evidence particularly that given by "Response to retention hypothesis" indicates that the whole sequence of events is found to be initiated by the retention of modified Low Density Lipoprotein[15,16], it was recongnized that lifestyle changes and the use of new pharmacologic approaches to lesser plasma cholesterol[17,18], Cardiac disease continues to be the main cause of death. The background trigger for atherosclerosis like structural changes of blood vessels is hidden , which has to be emphasized.

ATHEROSCLEROSIS

Atherosclerosis is a disease of arterial blood vessels. It is mainly due to accumulation of inflammatory cells such as macrophages, white blood cells promoted by small low density lipoproteins without adequate removal of fats and cholesterol from the macrophages by functional high density lipoproteins . It is commonly referred to as a "hardening" of the arteries leads to formation of numerous plaques within the arteries.

The lesions of atherosclerosis occurs mostly within the intimal layer of the artery wall. They inlcude[19-20],

- Fatty streak
- Fibrous plaque
- Complicated lesions
  - Plaque disruption
  - Athero thrombosis

FATTY STREAK

The process of atherogenesis begins in childhood with the development of fat, lipid rich lesions called fatty streaks. They are also found to contain macrophages, T lymphocytes, smooth muscle cells – each of these cells are found to be loaded with cholesterol. The lesions are yellowish and sessile in appearance and they cause little

obstruction of the affected artery. Lipid deposition does not lead to the complex lesions of atherosclerosis but progression of disease is associated with that a number of factors resulting the formation of fibrous plaque.

FIBROUS PLAQUE

It is otherwise called as fibromusculoelastic consists of proliferated smooth muscle cells,connective tissue with little lipid and that the deep core of lipid and cell debris results from insufficient blood supply, inflammation, and cell necrosis. A fully blown fibrous plaque consists of many smooth muscle cells lined by a dense connective tissue matrix often intermixed with numerous macrophages.

ADVANCED LESIONS – PLAQUE DISRUPTION AND ATHEROTHROMBOSIS

The typical advanced, complicated lesion contains a large necrotic core with a fibrous core, loaded with macrophages. The macrophages can from numerous proteolytic enzymes, including metalloproteinases – these enzymes cause the removal of fibrous cap – by plaque disruption and thinning of fibrous cap. The plaque disruption allow the lesion to get involved in thrombotic episodes that can lead to occlusive disease[21].

Thus, events atherosclerosis include, causes

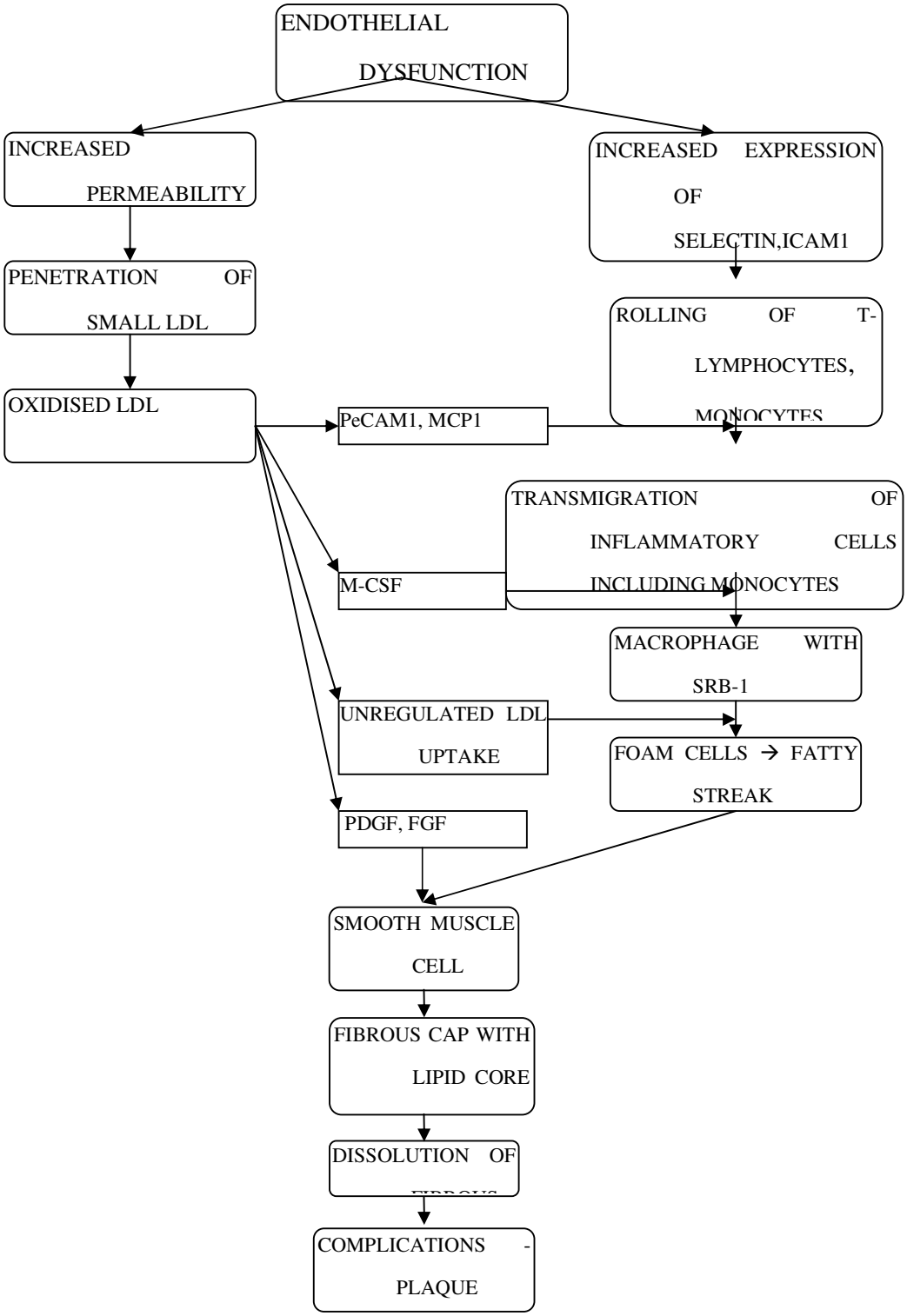
1. First, the rupure of atheromatous plaques, lead to stenosis of the artery therefore, an inadequate blood supply to the organ.
2. Second, formation of aneurysm occurs if the compensating artery enlargement process is extreme.

The rupture of the plaque exposes its thrombogenic contents into the lumen that will rapidly slow the blood flow, leading to death of the tissues fed within 5 minutes. This catastrophic event is known as infarction.

The clinical scenario of this catastrophic event depends on which artery is affected by this event.

1. Thrombosis of a coronary artery, causing myocardial infarction is called as Coronary artery disease.
2. Second most common is that caused due to thrombosis of carotid artery branches and inadequate blood supply to brain – which presents itself as stroke or transient ischemic attack.
3. Peripheral artery disease caused by insufficient blood supply to the legs, typically due to a combination of both obstruction s and aneurysm .
4. Arteries of the intestines, kidneys, legs, are also affected.

Fig 1. PATHOGENESIS OF ATHEROSCLEROSIS



HYPOTHESIS OF ATHEROGENESIS

It has been recognized in human for thousands of years. Long has discussed the development of clinic-pathologic correlations that allowed the formulation of a hypothesis relating the grading of atherosclerosis to the frequency of myocardial infarction and stroke[22]. Virchow proposed the idea that degenerative lesion of atherosclerosis some form of injury to the arterial wall related with the inflammatory responses. [23]. This idea was later customized by Antischkow[24] and the part of platelets and thrombus formation in atherosclerosis is included by Duguid[25]. The endothelial lining[26], of the artery is a key element in the maintenance of normal arterial function is distinguished by John French.

RESPONSE TO INJURY HYPOTHESIS

Endothelial cells provide thromboresistance surface that promotes the continuous flow of blood throughout the vascular tree [27,28,29,30,31]. Smooth muscle cell migration and proliferation is mediated by various cytokines. Small plasma lipoproteins are transported by endothelial cells into the arterial all[32]. The endothelium exhibits the thromboresistant characters by production of three factors. They are the cell surface glycoproteins and proteoglycans that form the surface coat of the endothelial cells, prostacyclin[30], and the most potent agent, nitric oxide[31]. Prostacyclin and NO are potent vasodilatory agents and

potent inhibitors of platelet aggregation. The hypothesis posits that some form of " injury" to the endothelium causes alteration in structure and function of the endothelial cells. So that the lipoproteins and inflammatory cells are more easily penetrate into the artery wall.

The alteration of endothelium is associated with overexpression of E, L, P selectin that appear to play a role in inducing rolling and attachment of monocytes and T lymphocytes to endothelium. This rolling is facilitated by the upregulation of ICAM 1 and VCAM 1 also. Another molecule formed by endothelium, PeCAM 1 has been shown to participate in interendothelial migration by the adherent leukocyte into the subendothelial space or intima of the artery. Thus, the earliest phase of the chronic, inflammatory response that has become recognized to be the hallmark of atherogenesis is represented by leukocyte adhesion due to the formation of these attachment and adherence molecules on the surfaces of the endothelium and the leukocytes[32-36].

A second event accompanying endothelial dysfunction is transmigration of lipoproteins particularly of small LDL particles, this transmigration places LDL in the subendothelial space which is virtually devoid of any antioxidant properties of the circulation, hence it gets oxidized. Oxidized LDL can act as chemotactic reagent. The monocyte

gets activated to macrophages, which express SR-B1 causing unregulated uptake of LDL particles, forming foam cells. Such a lesion with foam cells, activated inflammatory cells is called as fatty streak.

Oxidised LDL, foam cells, the activated macrophages, T-cells, produce various cytokines IL-1, TNF-α, Under the influence of these cytokines, endothelium, macrophages and T cells produce PDGF, FGF[58]. PDGF facilitates the movement and proliferation of smooth muscle cell. FGF stimulates the vascular smooth muscle cell to produce collagen and the various components of extracellular matrix together they form the fibrous cap. TNF-α induces apoptosis of foam cells causing exocytosis of its lipid content, which forms the lipid core. Such a lesion with lipid core, surrounded by activated T-cells, macrophages, platelets, lined by a fibrous cap is called as a stable atherosclerotic plaque. Thus oxidized LDL is not only toxic to the endothelium and the surrounding cells in the intima but also chemotactic for monocytes and can activate monocyte derived macrophages to produce growth factors and cytokines. Hence it may be the principal culprit in advancing the lesions of atherosclerosis.

All these lesions would be reversible and, reversed back to normal if the endothelial injury is self –limited. But when it is constant over period of many years, the lesion continues to progress, and becoming more complex. Because, the atherosclerotic plaque does not only have

smooth muscle cells but also macrophages, which are capable of producing metalloproteinases and TNFα, both of which cause necrosis and digestion of the fibrous cap, this loss of fibrous cap is responsible for the complications of atherosclerosis, namely plaque rupture. This plaque rupture exposes the subendothelial extracellular matrix to the factors of coagulation in the circulation initiating the intrinsic pathway of coagulation – this is responsible for atherothrombosis.

FACTORS INFLUENCING ATHEROGENESIS

UNMODIFIABLE RISK FACTORS

1. Age
2. Male sex
3. Socioeconomic status

MODIFIABLE RISK FACTORS

1. Cigarette smoking
2. Alcoholism
3. Insulin resistance & hyperglycemia
4. Hypertension
5. Obesity
6. Oxidative stress
7. Abnormal lipid profile

a. High total and LDL cholesterol

b. Low HDL cholesterol

c. High triglycerides

d. Lipoprotein(a)

8. Physical inactivity

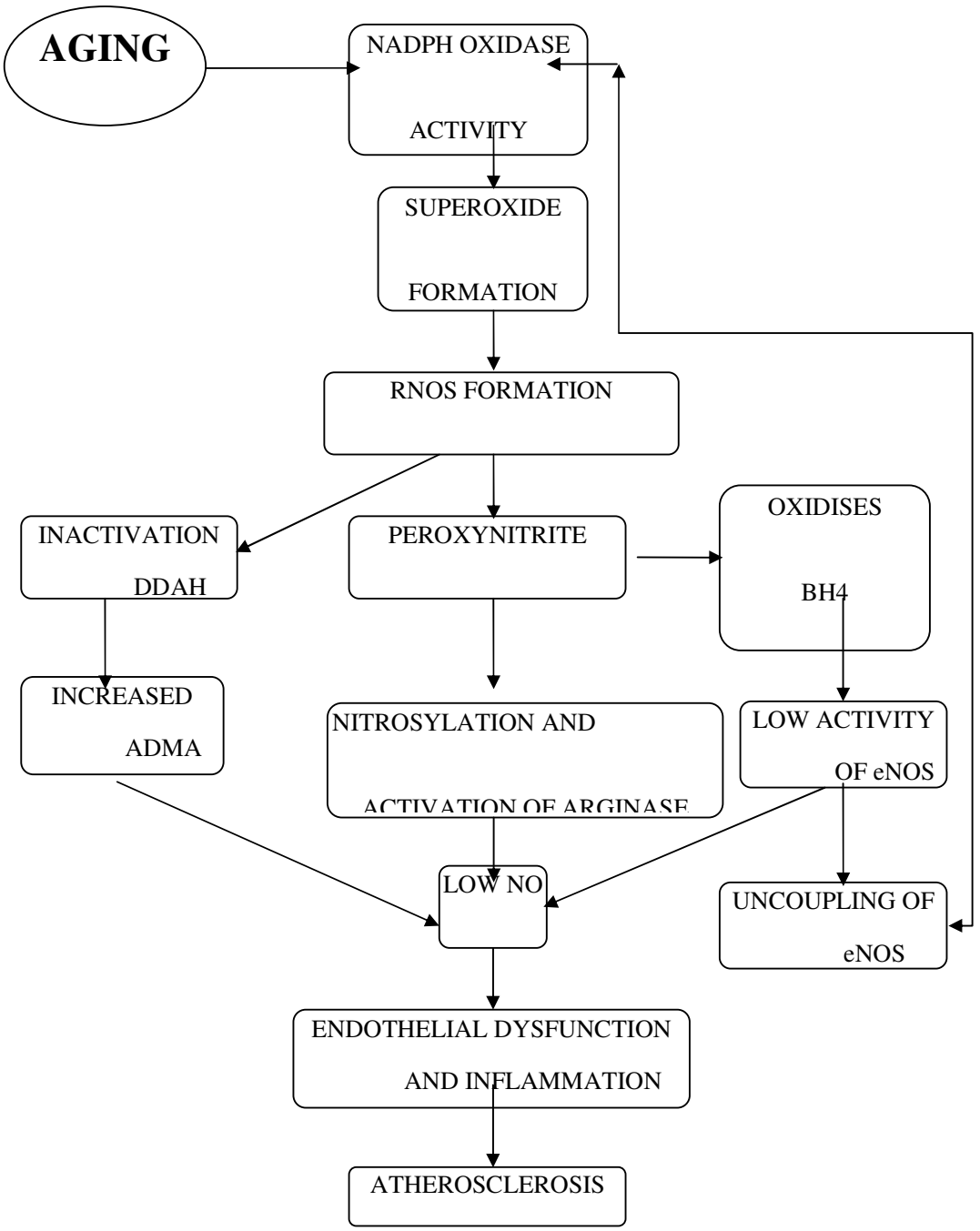
Age

In many epidemiologic surveys, age remains one of the strongest predictors of disease. The majority of patients with atherosclerotic coronary heart disease are more than 65 years old. Older patients have higher mortality and more complications. Age related changes in the cardio vascular system and other organs make it reasonable to assume that aging per se constitutes a major reason for the increased morbidity and mortality in older persons. These age related changes include diastolic dysfunction, degenerative changes in the conduction system, reduced responses to catecholamine and sympathetic stimuli.

Male sex

The relationship of gender to the development and prognosis of atherosclerotic coronary heart disease is complicated[37]. The powerful protective effect of the premenopausal state in preventing and postponing the condition is fully appreciated; women tend to develop atherosclerotic coronary heart disease approximately 10 years later than men. In women

Fig 2 ROLE OF AGING IN ATHEROSCLEROSIS



under the age of 60 epicardial coronary atherosclerosis is uncommon[38]. Complications are fewer in women after the onset of angina, but they may be more frequent after myocardial infarction[39,40]. However there are multiple reports indication a gender bias in reference to the use of diagnostic and therapeutic procedures, but interpretation is complicated by the possibility of overuse and overtreatment in low risk men[41-45]. The point has been made clearly. Atherosclerotic coronary heart disease manifest as angina, infarct, and sudden death is as common in women after age 60 as it is in men.

This gender dependent differential risk is attributed to the protective function exerted by estrogens. Recently, a study of estrogens and their effect on smooth muscle cells and the other elements of atherogenesis showed that estrogens have an antiproliferative effect on smooth muscle cells and can be protective to the endothelium in relation to stimulation by growth factors, cytokines and other agents. Estrogen is not only antiproliferative for smooth muscle but also has been shown to be capable of modulating acetylcholine mediated dilation of atherosclerotic coronary arteries[46].

Family history of Early – Onset CHD

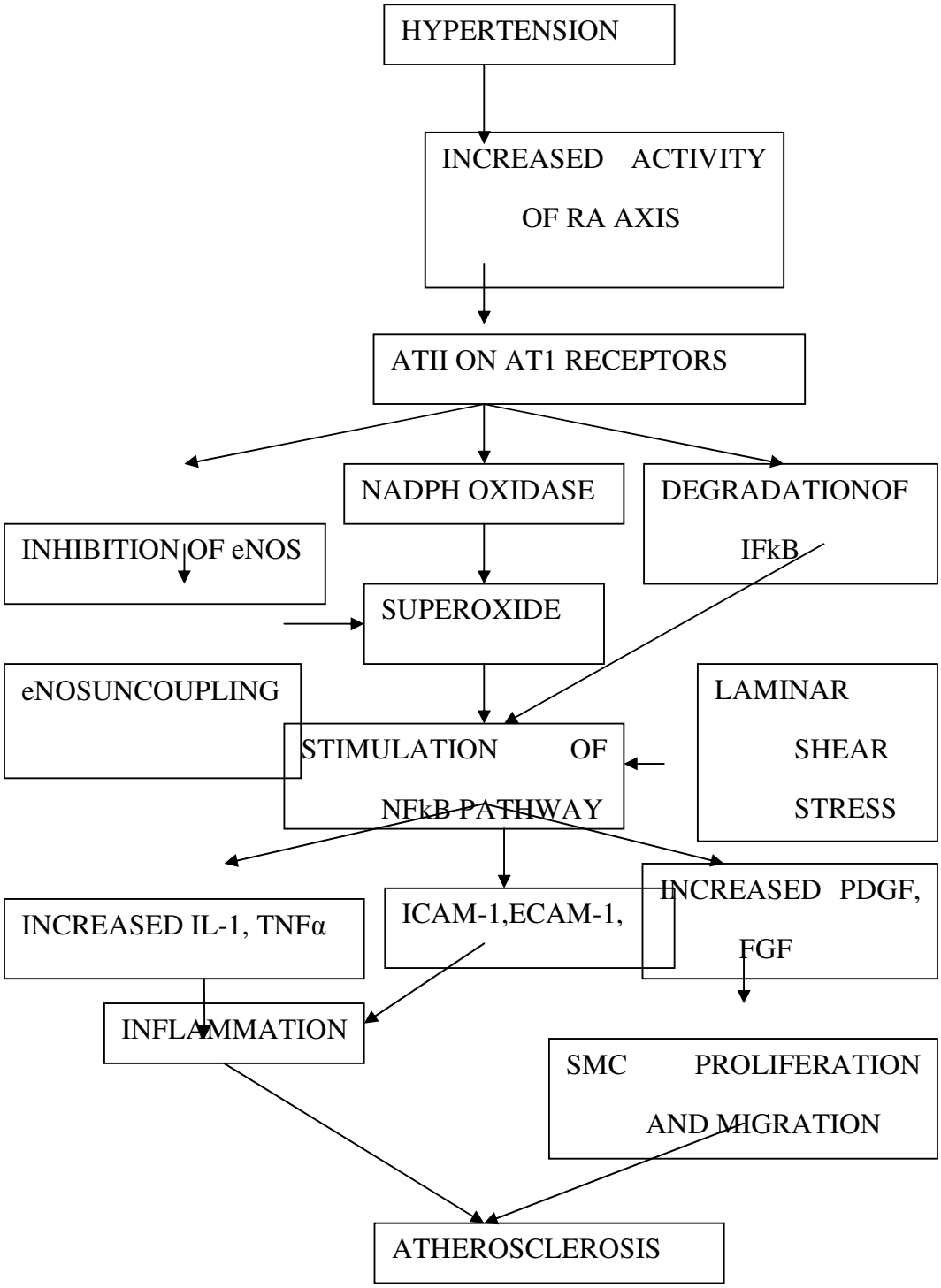
Over 35 case-control and prospective studies have consistently indentified an association between CHD and a history of first degree relatives with early onset CHD[47]. This risk generally persists even

after adjustment for other risk factors. The family history most predictive of coronary disease is that of a first degree relative developing CHD at an early age. Positive family history[70] denotes when male with disease at 55yrs or less and female with onset at 65yrs or less. The larger the number of relatives with early onset of CHD or the younger the age of CHD onset in the relative, the stronger the predictive value[49,50].

Socioeconomic status

A consistent relationship has been devised between lower socioeconomic status and atherosclerosis. There has been the perception that conventional risk factors cluster in lower socioeconomic groups and that this phenomenon can explain the increased incidence of atherosclerotic coronary heart disease[51]. But, only 50% of atherosclerotic coronary heart disease can be explained by known risk factors. The socioeconomic status proved to be independent predictors in patients with established atherosclerotic coronary heart disease[52]. Although no simple relationship between socioeconomic status, risk for cardiovascular disease and long term outcome for manifest atherosclerotic coronary heart disease can be devised, the evident is consistent and persuasive that lower socioeconomic status is an independent and significant determinant of long-term outcome.

Fig 3. EFFECT OF HYPERTENSION ON ATHEROSCLEROSIS



Hypertension

Epidemiological studies have established found that both systolic and diastolic bloodpressure have a positive and graded correlation to CHD[53,54.55]. The risk imposed by hypertension is increased substantially when other risk factors are present. Hypertension clusters insulin resistance, hyperinsulinemia, glucose intolerance, dyslipidemia, left ventricular hypertrophy and obesity and occurs in isolation in fewer than 20% of individuals[56].

The potential mechanisms by which hypertension may cause impaired endothelial function include increased endothelial permeability to lipoproteins, increased adherence of leukocytes, increased oxidative stress, and hemodynamic stress that may trigger acute plaque rupture, all these mediated by initiation of NF-kB pathway and inactivation of eNOS enzyme.

Hyperglycemia

Hyperglycemia is an independent risk factor for CHD, increasing the risk by two to three times for men and three to five times for women[57]. CHD is the leading cause of death in diabetic patients and approximately 25% of MI survivals have diabetes[58]. The CHD risk for a premenopausal diabetic woman is similar to the risk of a nondiabetic man,hence diabetes abolishes the protective effect of being a

premenopausal female[59]. Diabetic women have twice the risk of recurrent MI compared with diabetic men[60]. The greater risk of CHD in diabetic women compared to diabetic men may be explained in part by the greater adverse effect of diabetes on lipoproteins in women[61].

Potential mechanisms by which hyperglycemia may cause atherosclerosis include impaired endothelial function, glycation of LDL, enhanced lipoprotein oxidation, increased fibrinogen, increased platelet aggregation, impaired fibrinolysis, increased small LDL. All these are attributed to the increased flux of glucose into glycolysis (glucose uptake and hexokinase activity in endothelium is insulin independent), as a result there is an increased NADH/NAD ratio, causing increased flux of electrons through electron transport chain, producing superoxide radicals. This causes DNA damage and the resultant ADP ribosylation of proteins inhibit glyceraldehyde 3-phosphate dehydrogenase of glycolysis, causing accumulation of glyceraldehyde 3- phosphate and its precursor fructose 6-phosphate. The former cause activation of protein kinase C pathway through DAG – protein kinase C pathway stimulates the production of various cytokines and thereby stimulates inflammation. Fructose 6 phosphate stimulates hexosamine pathway, thereby stimulate N- glycosylation of many proteins like eNOS and inhibition of them, this causes NOS uncoupling and the resultant oxidative stress further aggravates the condition. Furthermore, there is increased formation of advanced glycation end product, which on

binding to receptor for advanced glycation end products is found to stimulate NF-kB pathway, causing all the features of atherosclerosis.

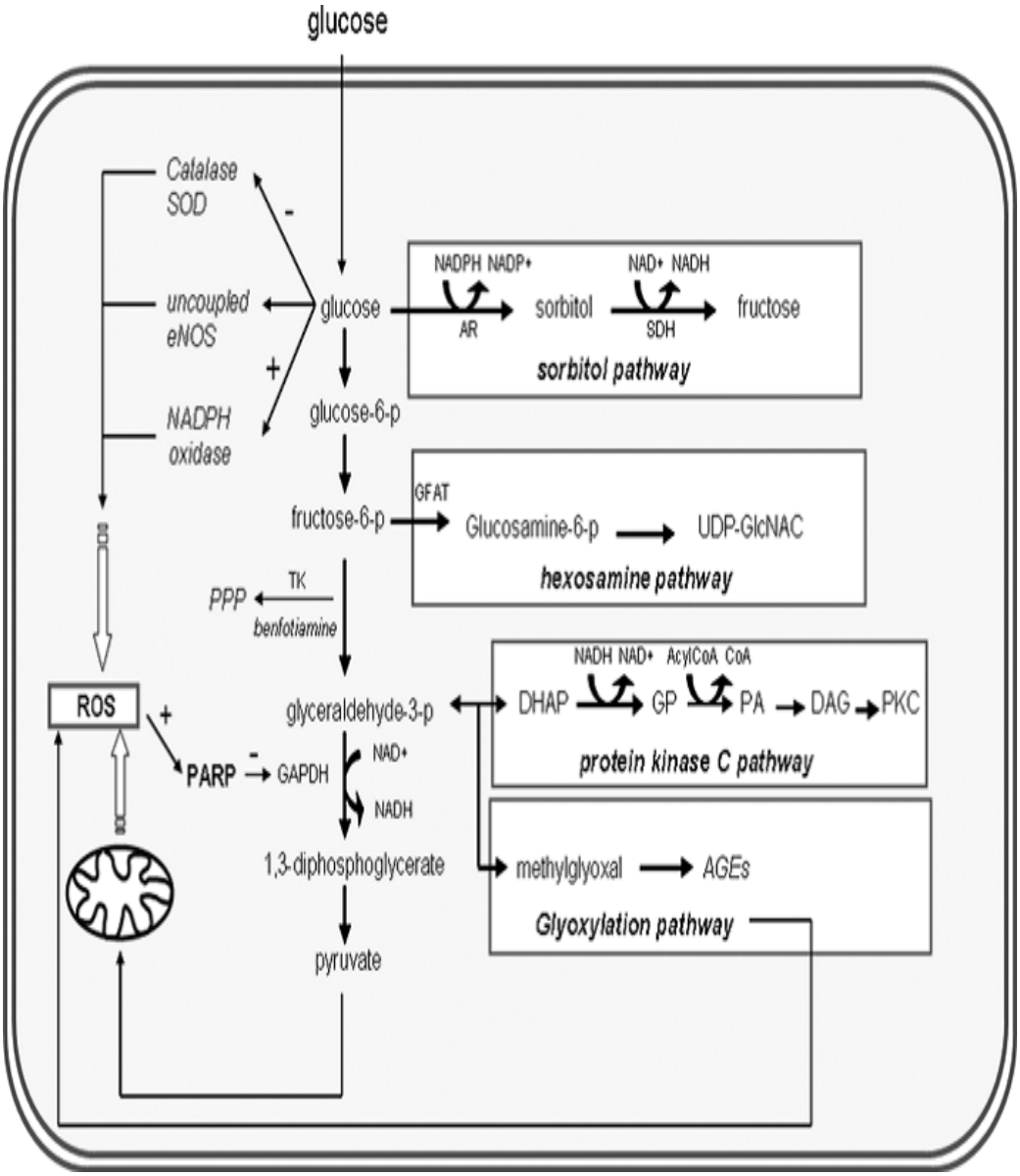
Insulin resistance and hyperinsulinemia

Coronary risk factors are resistance to insulin, hyperinsulinemia , hypertension, diabetes, hypertriglyceridemia, low HDL, predominance of small LDL, and elevated plasminogen activator inhibitor concentration[62,63]. Hyperinsulinemia may raise blood pressure through sympathetic nerve stimulation and/or renal sodium retention. Insulin sensitivity is associated with endothelial nitric oxide production in healthy persons providing a clue as to how insulin resistance may promote CHD directly[64]. Furthermore, hyperinsulinemia has been found in a prospective study to be an independent risk factor for CHD in nondiabetic men after adjusting for body weight, blood pressure and dyslipidemia[65].

Physical inactivity

Physical inactivity roughly doubles the risk of CHD. Moderate intensity exercise decreases coronary atherosclerosis and widens coronary arteries in monkeys fed on atherogenic diet compared with monkeys fed the same diet but forced to be inactive[66].There is a slow development of angiographically defined coronary atherosclerosis in human with physical activity[67]. Men with physical fitness have reduce the risk of CHD[68]. The

Fig 4. PATHOGENESIS OF ATHEROSCLEROSIS IN DIABETES MELLITUS



risk of MI and sudden cardiac death is greatest during exercise, leading some to question the benefits of exercise[69]. The overall risk of myocardial infarction and sudden cardiac death, is however low among those who exercise regularly. The greatest reduction in risk is between sedentary individuals and those who do regular moderate intensity activity.

Physical activities may lessen CHD risk include by rising HDL, decreasing hypertension and obesity, reducing insulin resistance, decreasing platelet accumulation and increasing fibrinolysis[68].

Obesity

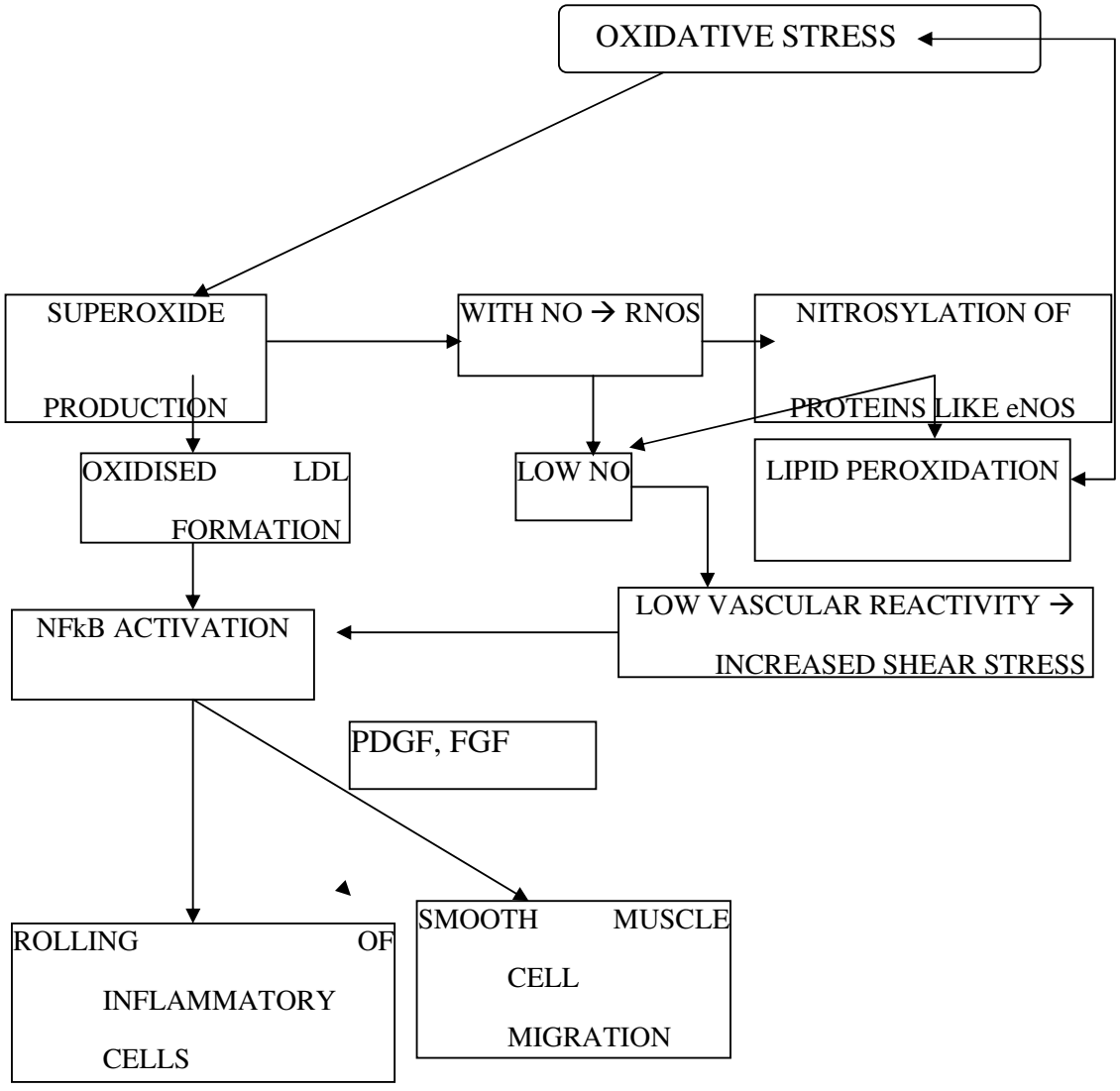
Obesity promotes insulin resistance, hyperinsulinemia, hypertriglyceridemia, low HDL cholesterol, and LVH[70,71]. Many observational studies have found that obesity strongly and positively correlates with the risk of CHD in univariate analysis. In multivariate analysis, when controlling statistically for risk factors such as hypertension, diabetes, and dyslipidemia, obesity is not found to be an independent risk factor. Rather it reflects that much of the adverse consequences of obesity are mediated through resultant metabolic risk factors acting as pathological links in the causal pathway. Nevertheless, some large prospective observational studies of long duration indicate that obesity also increases coronary and cardiovascular mortality in men and women[72-74]. The deposition of fat in central portions of the body

predicts CHD in men independently of body-mass index and other major risk factors[75]. Weight loss improves insulin sensitivity and glucose disposal; reduces blood pressure, triglycerides and LVH; and increases HDL cholesterol[70,71].

Oxidative stress

Over production of reactive oxygen species has been concerned to play a major role in a number of cardiovascular pathologies. ROS are generated in vascular cells by NADPH oxidases, uncoupled eNOS, and as a product of mitochondrial respiration[37]. If this production goes unbalanced, it leads to exacerbation of pathophysiological processes. Superoxide radicals are found to cause oxidative modification of LDL. Oxidised LDL, by activating NF-kB pathway of inflammation is found to mediate the increased production of IL-1, increased expression of ICAM, both of which mediate the rolling of inflammatory cells. Furthermore there is increased production of PDGF and FGF, both of which cause smooth muscle cell proliferation and migration. This cycle is reinforced by the decreased production of NO, because the superoxide cause nitrosylation of eNOS and thereby it inhibits the enzyme activity. The decreased NO causes increased vascular reactivity and the resultant shear stress will further stimulate NF-kB pathway. Thus, commencement and development of atherosclerosis is influenced by oxidized LDL[76-79] .

Fig 5, ROLE OF OXIDATIVE STRESS IN ATHEROSCLEROSIS



Cigarette smoking

Strong dose relationships between cigarette smoking and coronary heart disease have been observed in both sexes. Cigarette smoking increases the risk two to three fold and interacts with other risk factors to multiply risk. Pathophysiological studies have identified a panoply of mechanisms through which cigarette smoking may cause CHD. Smokers have increased levels of oxidation products, including oxidised LDL. Cigarette smoking also lowers the cardioprotective levels of HDL. These effects, along with direct effects of carbon monoxide and nicotine, produce endothelial damage. Possibly, through these mechanisms, smokers have increased vascular reactivity[80]. Cigarette smoking is also related to increased levels of fibrinogen[81] and increased platelet aggregability[82]. Thus cigarette smoking paves way for atherosclerosis by inducing oxidative stress and by altering coagulability.

Dyslipidemia

Total cholesterol and LDL cholesterol

Many studies have identified a permanent, graded and direct association between serum cholesterol and CHD occurrence.[83]. The level of total and LDL cholesterol interacts with other risk factors to multiply risk[84]. Elevated LDL cholesterol levels have been related to

recurrent events and CHD death in patients with established CHD[85]. The progression of atherogenesis is mediated by increased LDL cholesterol levels. Elevated cholesterol concentrations in the plasma lead to an increased release of LDL particles into the arterial wall. Their oxidation leads to the formation oxidized LDL which act as chemoattractants[86]. LDL is also a potent mitogen for smooth muscle cells; progressive growth of atherosclerotic plaques can be halted by lowering of LDL cholesterol levels. Atherosclerotic plaques with a large lipid core and numerous lipid filled macrophages are prone to rupture[87].

Furthermore, small dense LDL is felt to be more atherogenic[88]. Possible explanation for this is that when a person has more of small LDL particles, for given cholesterol content, the number of LDL particles will be more, and an LDL receptor can accept only one LDL particle at a time and hence the rate of metabolism of LDL is decreased, causing accumulation of LDL in the plasma. The second reason for the same is the endothelium will be more permeable to small LDL particle when compared to a normal LDL.

Triglycerides

The relationship between triglycerides and CHD has been less clear. This relationship usually disappears after adjustment for other risk factors such as HDL cholesterol, obesity and diabetes[89]. Hypertriglyceridemia however has been found to be an independent risk

factor in women[90]. Several mechanisms have been proposed to explain the triglyceride- CHD association. First, some patients with hypertriglyceridemia have a predominance of small, dense LDL particles. Second, fasting hypertriglyceridemia may be a marker of exaggerated postprandial hyperlipidemia, which may promote the uptake of atherogenic triglyceride rich lipoprotein remnants by endothelial cells[91]. Finally, serum triglyceride levels are strongly related to fibrinogen and factor VII in numerous epidemiological studies[92]. Therefore, number of mechanisms, direct and indirect link serum triglycerides and CHD.

Low HDL cholesterol

There is indirect association between HDL cholesterol levels and the frequency of CHD[93]. The ratio of total cholesterol to HDL cholesterol is a superior interpreter of CHD than the HDL cholesterol level alone[93]. Two important mechanisms by which HDL is thought to play a protective role against atherosclerosis are reverse cholesterol transport and inhibition of LDL oxidation.

MMP-2

MMP-2 is a zinc and calcium dependent endopeptidases[94]. MMP-2 has MW of 72KDa, is also known as type IV collagenase that degrades native type IV, V, VII, and X collagen[95] . Type IV is a main

component of the basement membrane. MMPs are zinc dependent endopeptidase, able to cleave the ECM. ECM plays a key role for the proper function of different organs of the human body including heart and blood vessels. MMPs cause proteolytic destruction of ECM[96]. Because of its presence everywhere in the body , it has various functions.[97] Gelatinases composed of the 72KDa MMP-2 and 92KDa MMP-7. Gelatinase A is an other name of MMP-2.

All MMPs are produced in inactive structure. They are produced as proenzymes and need extracellular activation. For its activation MMPs need zinc . The activation steps consist of three different mechanisms:1. stepwise activation, 2.cell surface activation and 3. intracellular activation.

These enzymes are produced in low amounts by normal cells linked with normal tissue remodeling such as healing of wounds, implantation, invasion of trophoblasts, and angiogenesis . MMPs are often over expressed in malignant tumors .

The matrix metalloproteinase family.

MMPs-MMP-1

Name-Fibroblast

Subfamily-Interstitial

Main substrate -Fibrillar collagen

MMPs-MMP-8

Name-Neutrophil

Subfamily-- Collagenase

Main substrate-Fibrillar collagen

MMPs-MMP-13

Name--- Collagenase-3

Subfamily-- Collagenase

Main substrate-Fibrillar collagen

MMPs-MMP-18

Name--- Collagenase-4

Subfamily-- Collagenase

Main substrate-Fibrillar collagen

MMPs-MMP-2

Name--- Gelatinase

Fig 6.RIBBON DIAGRAM OF HUMAN PRO MMP-2 AND ACTIVE MMP-2

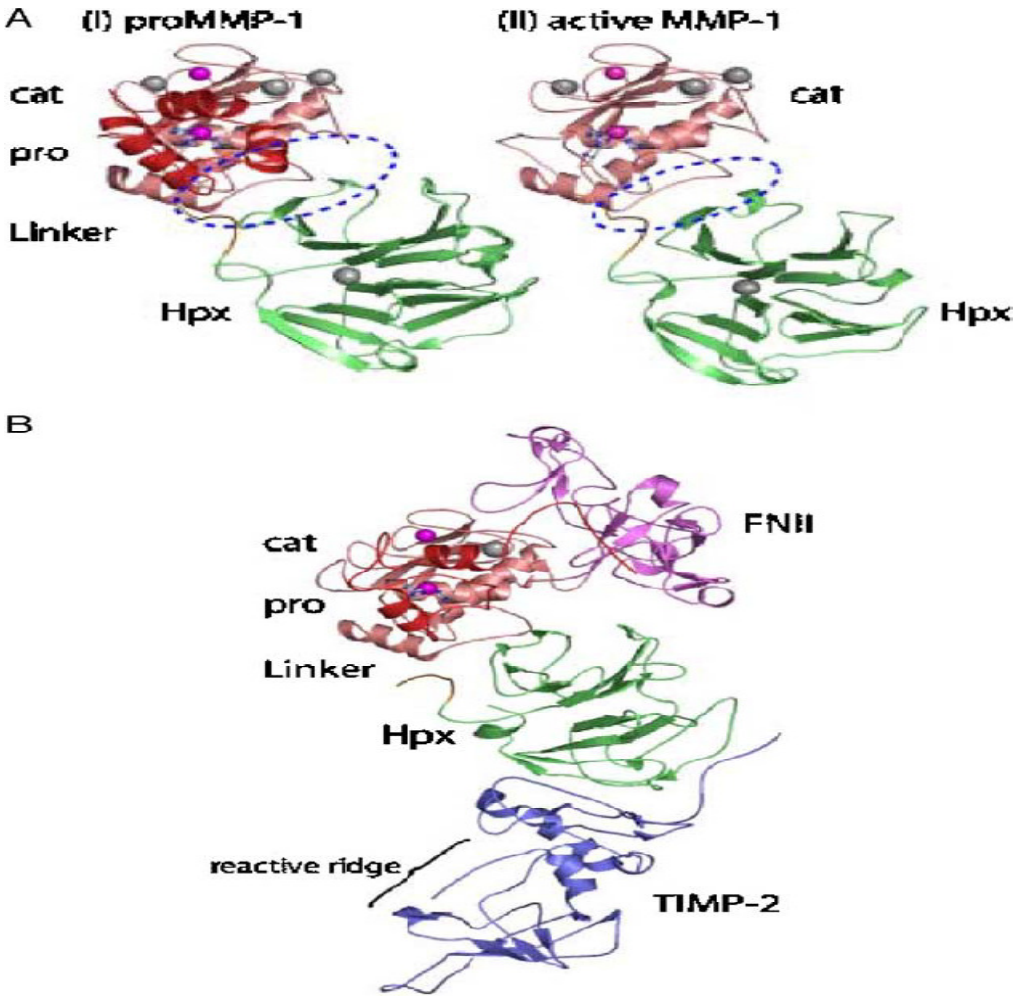


Fig. 6. (A) Ribbon diagram of human proMMP-2 and active MMP-2. The pro-domain is shown in red, catalytic domain in pink, the linker region in yellow, the hemopexin domain in green, zinc ions in purple, calcium ions in grey. The dotted circle indicates the region where the catalytic and hemopexin domains intereact. proMMP-2 has a larger area of
Contact sites than MMP-2. This results in the active form has an ‘‘open’’ configuration compared the ‘‘closed’’ configuration of proMMP-2. (B) Ribbon structure of the complex of proMMP-2 and TIMP-2. Domains of proMMP-2 are shown as in (A) and the finbronectin type II (FNII) motif is in purple.

Subfamily- Gelatinase A

Main substrate- Gelatin,Type IVcollagen.Fibronectin.Elastin,Laminin

MMPs-MMP-9

Name--- Gelatinase

Subfamily- Gelatinase B

Main substrate- Gelatin,Vitronectin,.Fibronectin.Elastin,Laminin

MMPs-MMP-3

Name--- Stromelysins

Subfamily- Stromelysins-1

Main substrate-Fibronectin,TIMP-2

MMPs-MMP-10

Name--- Stromelysins

Subfamily- Stromelysins-2

Main substrate- Fibronectin,TIMP-2

MMPs-MMP-11

Name--- Stromelysins

Subfamily- Stromelysins-3

Main substrate- Gelatin,.Fibronectin.Elastin Laminin,Aggrecan.

MMPs-MMP-7

Name--- Matrilysin

Subfamily- Stromelysins

Main substrate- Gelatin,.Fibronectin.Elastin ,Vitronectin,Aggrecan

MMPs-MMP-12

Name--Metallo- Elastases

Subfamily---- Elastases .**Main substrate-** Gelatin,.Fibronectin,Elastin, Vitronectin,Proteoglycan,Collagen-IV

MMPs-MMP-14

Name—MT1-MMP

Subfamily---- Membrane type MMPs

Main substrate- Pro MMP-2,Pro collagenase

MMPs-MMP-15

Fig 7.DOMAIN STRUCTURE FOR MAJOR CLASSES OF MMPs

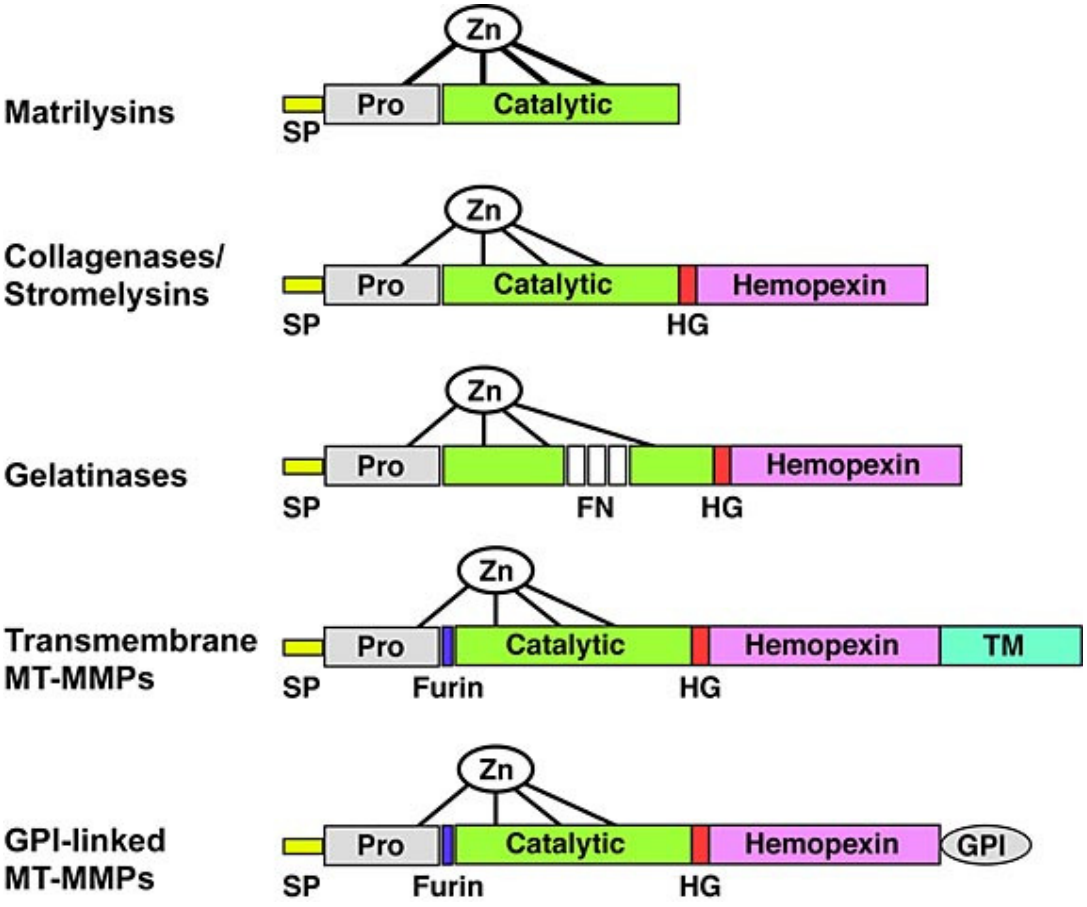
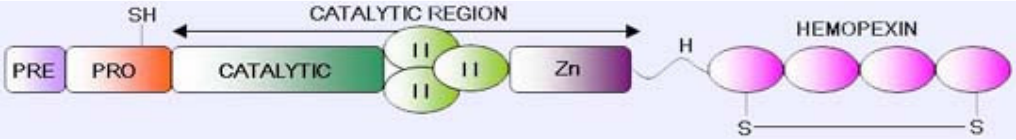


Figure 7. Domain structure for the major classes of MMPs. Major domains include the signal peptide (SP), prodomain (Pro), catalytic domain with the active site zinc (Zn) bound to cysteine residues within this domain and "cysteine switch-residue" in the prodomain, the hinge domain (HG), the hemopexin domain, and in some cases either a transmembrane domain or GPI-anchor domain (GPI). A furin cleavage site between the prodomain and the catalytic domain is found in some MMPs. In the gelatinases, fibronectin-like type II repeats (FN) are also present

Fig 8. DOMAIN STRUCTURE OF MMP-2



Domain structure of the MMP2.

- **Pre: signal sequence;**
- **Pro: propeptide with a free zinc-ligating thiol (SH) group;**
- **Zn: zinc-binding site;**
- **II: collagen-binding fibronectin type II inserts;**
- **;**
  The hemopexin/vitronectin-like domain contains four repeats with the first and last linked by a disulfide bond.

- **H: hinge region;**

Name—MT2-MMP

Subfamily---- Membrane type MMPs

Main substrate- Pro MMP-2,

MMPs-MMP-16

Name—MT3-MMP

Subfamily---- Membrane type MMPs

Main substrate- Pro MMP-2,

MMPs-MMP-17

Name—MT4-MMP

Subfamily---- Membrane type MMPs

Main substrate- Nil.

Other MMPs-MMP-19

MMP-20(Enamelysin),Substrate-Amelogenin.

SYNTHESIS OF MMP-2

MMP-2 are produced by endothelial cells, smooth muscle cells, and fibroblasts. Oxidative stress which is involved in cardiovascular

disease, can stimulate MMPs production and activation[98]. MMP-2 is the principal matrix metalloproteinase secreted by smooth muscle cells. Most MMPs are produced as inactive zymogens. The majority of MMPs contain propeptide domain with a unique and highly conserved sequence of cysteine ('cysteine switch') is capable of binding zinc in the catalytic domain, and making the enzyme inactive[99]. Interruption of the cysteine- zinc bond and deletion of the propetide domain activates the catalytic domain. It also needs calcium ions for its activation . The C- terminal hemopexin like domain has a role in substrate binding[100]. Pro MMP-2 contains 631 aminoacid residues. Active MMP-2 contains 80 aminoacid residues.

ACTIVATION OF MMP-2

Activation of inactive enzyme can occur intracellularly, at the cell surface, and in the extra cellular space through the activation by previously activated MMPs through a course called stepwise activation[101]. The signals for endoproteolytic cleavage for furin via trans –Golgi network are considered to be from these MMPs which contain a motif of basic aminoacid sequence present in upstream of the catalytic domain[102,103].

At the cell surface Pro-MMP-2 binds with MT_1-MMP and TIMP-2, and the neighbouring MT_1-MMP cleave Pro-MMP-2 at the prodomain[104].

Fig 9.ACTIVATION OF PRO MMP-2

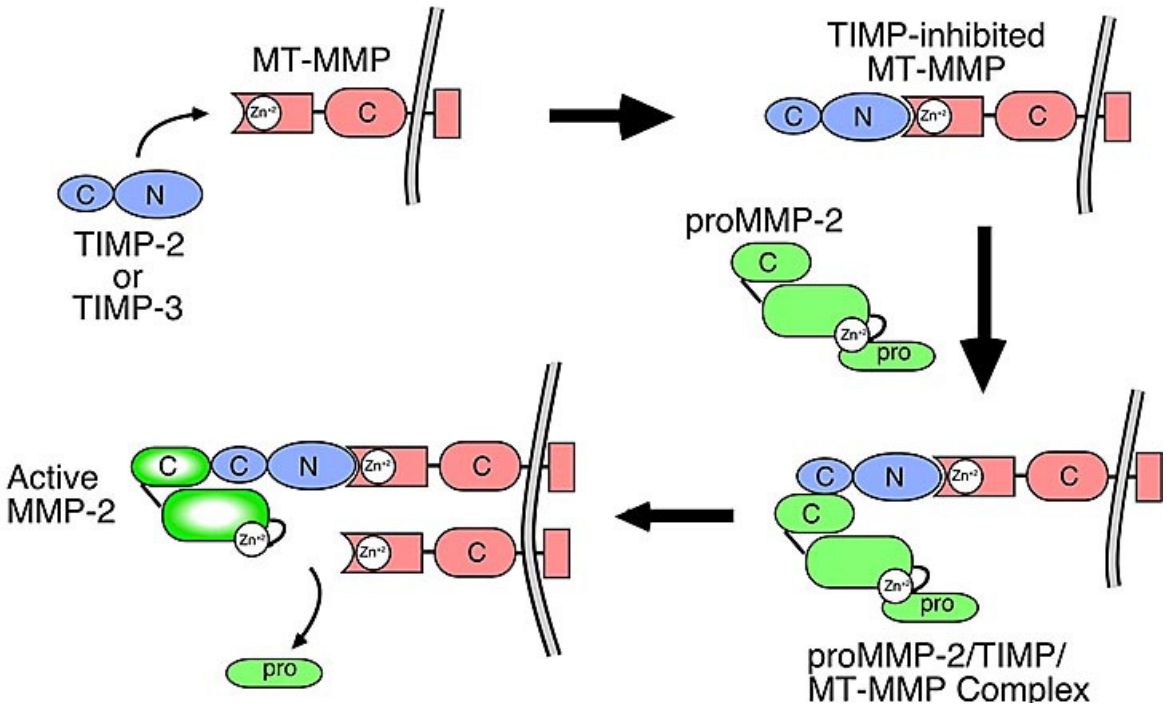


Figure 9. Simple model illustrating the basic steps of proMMP-2 activation through the formation of a proMMP-2/TIMP/MT-MMP ternary complex. In this model, TIMP-2 or TIMP-3 binds the catalytic domain of MT1-MMP or MT3-MMP forming a TIMP/MT-MMP complex on the cell surface. While this inhibits the activity of the occupied MT-MMP, TIMP-2 and TIMP-3 retain their ability to bind proMMP-2 effectively recruiting proMMP-2 to the cell surface. Once on the cell surface, an adjacent TIMP-free MT-MMP cleaves and activates proMMP-2.

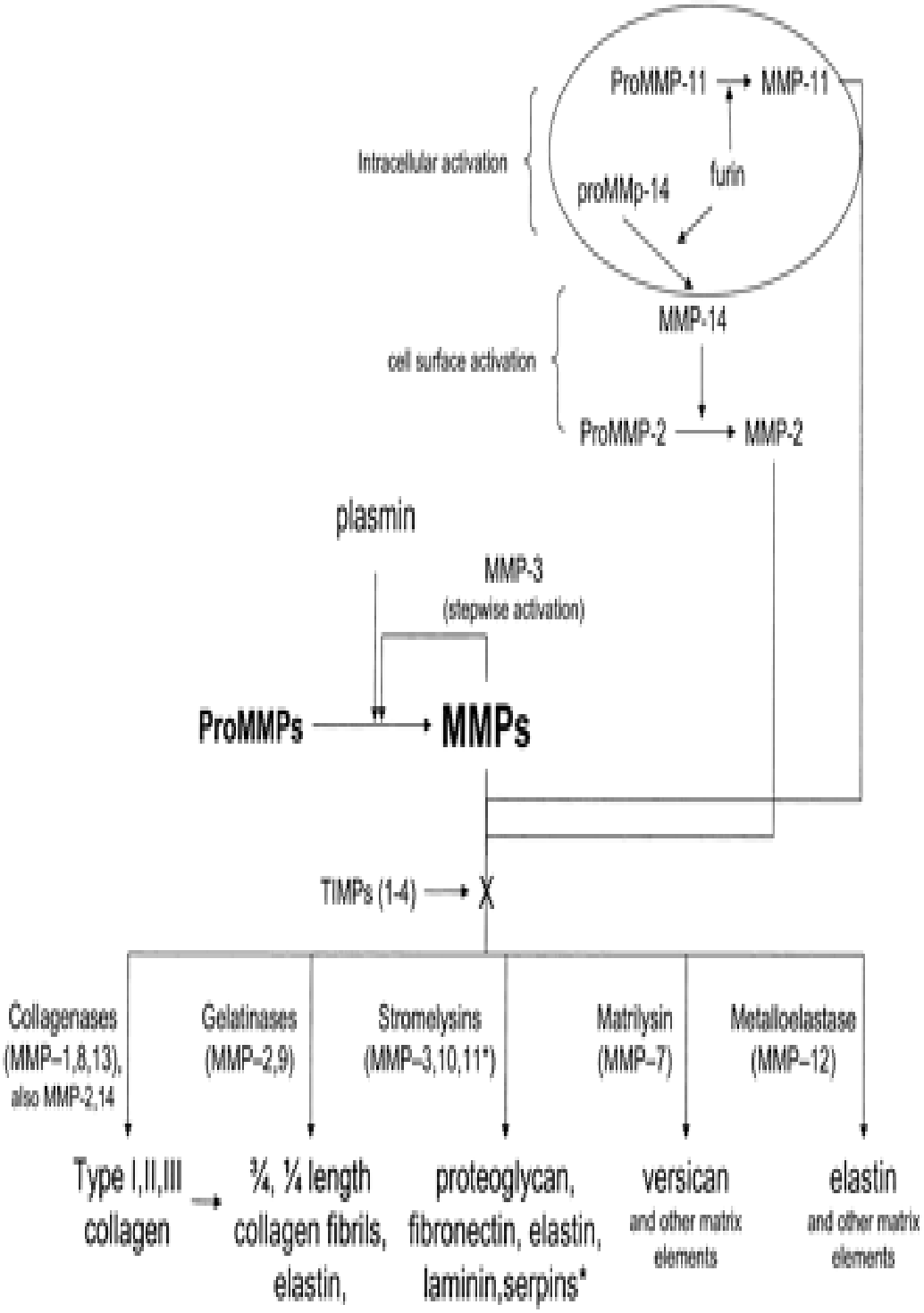
The integrin binds to the hemopexin domain of Pro-MMP-2 is considered to be the initiation of activation. Many integrins including ∝2 β1 and ∝2 β3 are involved. Some proteases, mainly plasmin, is the major contributor for extra cellular stepwise activation via cysteine switch[105]. Reactive oxygen compounds react with thiol groups and activate Pro-MMP-2 are thought to be via the 'cysteine switch' mechanism[106].

Thrombin, and factor Xa are also involved in activation of Pro-MMP-2. Activated MMP-2 activates Pro-MMP-9 and MMP-12 thereby causing a cascade-like effect on MMP activation[107]. MMP activity is also regulated by tissue specific inhibitor of metalloproteinases [108](TIMP-1, TIMP-2, TIMP-3, TIMP-4).

FUNCTIONS

MMP-2 has numerous functions including embryonic development, cell migration, wound healing, tissue resorption and angio genesis[109]. All these are due to its ability to modify the structural integrity of tissues. MMP-2 also involved in osteoblast bone formation and inhibits osteoclastic bone resorption[110]. Incresaed MMP -2 expression is found in the pathologic conditions, such as rupture of atherosclerotic plaques and following acute coronary syndromes[111].

Fig 10. INTRACELLULAR ACTIVATION OF PROMMP-2



ECM plays a key role for the proper function of different organs of the human body including heart and bloodvessels..

The main function of MMP-2 is destruction of proteins in ECM such as type IV, V, VII, IX and X collagen.

REGULATION OF MMP-2 ACTIVITY

There are three different levels for MMP-2 regulation. 1. Gene transcription, 2. Post translational activation of zymogens and 3. Interaction of secreted MMPs with inhibitors[112]. Inter relation of transcription factors, co-activators and co-repressor proteins with cis-acting elements in the promoter region of MMP-2 gene involve at the level of transcription. It is predominantly via prostaglandin E2 -cAMP dependent pathway. G proteins have been concerned to be involved[113].It is stimulated by inflammatory cytokines, hormones, and growth factors, such as interleukin-1β (IL-β), IL-6, tumor necrosis factor[114 – 117]. Hypoxia, more than 24 hrs, increases expression of mRNA for MMP-2.

Transcriptional regulation of MMP-2

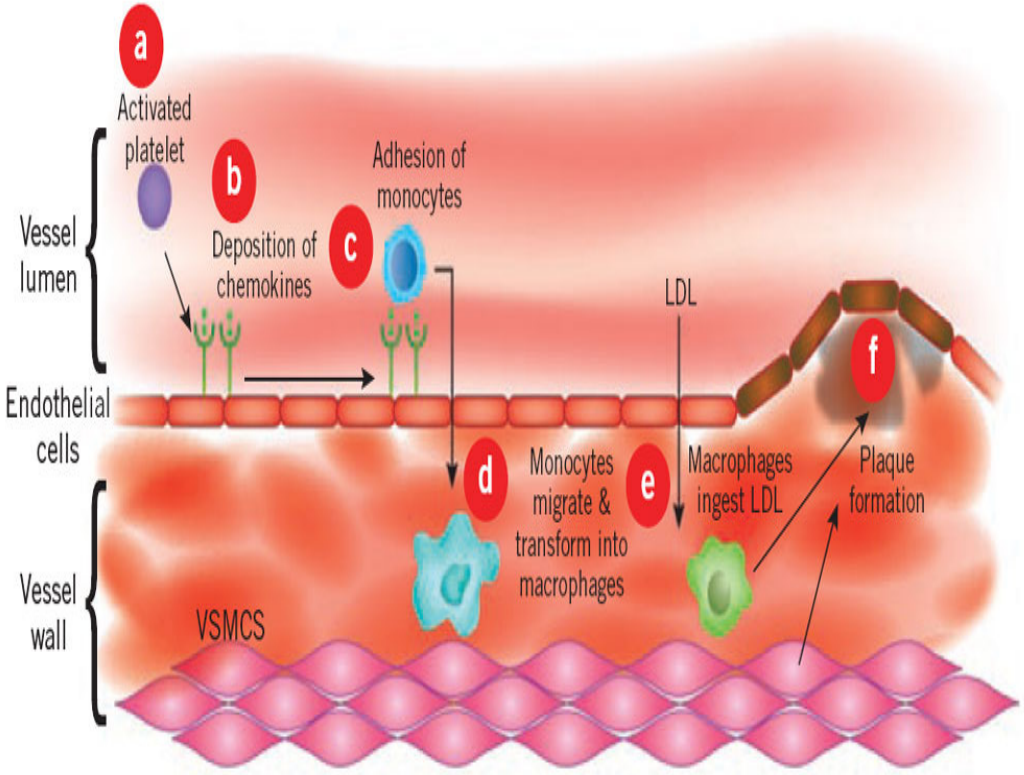
Indomethocin, corticosteroids, and interleukins 4[118,119,120]reduce gene expression. Their inhibition is due to addition of PGE2 or cAMP. PPARγ released from vascular smooth muscle cells and macrophages decreases MMP expression[121] .T to C alteration at a site – 1306 in

the promoter region of MMP-2 gene influences expression. This polymorphisms have been linked with cardio vascular disease.

ROLE OF MMP-2 IN ATHEROSCLEROSIS

MMPs cause plaque progression of plaque make it into vulnerable , and more prone for rupture. Inflammation, is a hall mark of atherosclerotic plaque. Atherosclerotic lesion develops in the course of a chain of highly specific cellular and molecular inflammatory response of the vessels wall to an initial injury. The initial lesion, principally producing dysfunction of endothelium. Endothelium tries to neutralize this initial damage but when it persists the inflammation is characterized by penetration of leukocytes, lymphocytes, macrophages, and smooth muscle cells into the vessel wall along with lipids. These abundantly produced and activated MMP-2 assist the access of smooth muscle cells, monocytes, macrophages through endothelial cell layer by degrading the basement membrane. Simultaneously along with the ingestion of cholesterol, the development of plaque in the vessel wall is initiated and the plaque grows[122,123] resulting reversible fatty-streak lesion. In the sub intimal space, MMP-2 activity further boosted by inflammatory cytokines such as interleukin-1, TNF-$\propto$, and oxidized LDL resulting non reversible lesion consist of activated macrophages, foam cells, T lymphocytes,

Fig 11.ROLE OF MMP-2 IN ATHEROSCLEROSIS



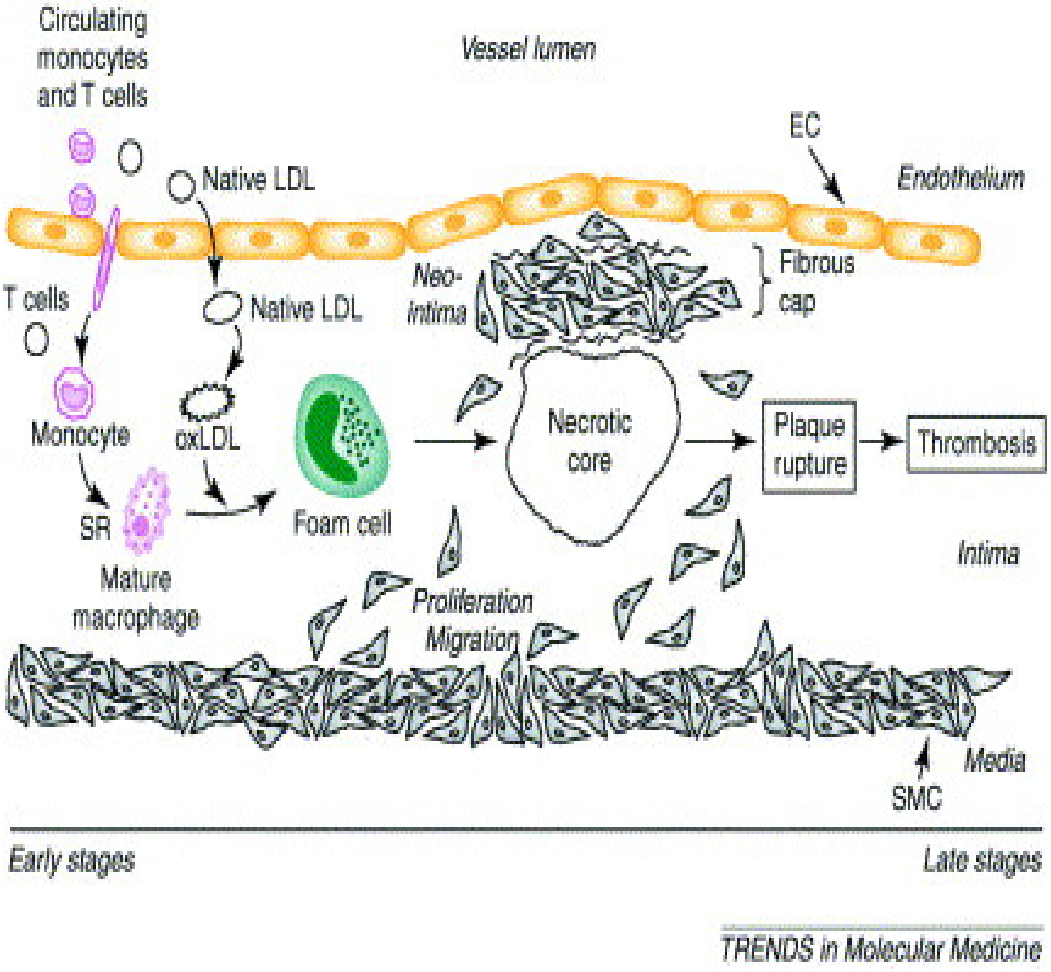
mast cells and other such cells around a necrotic lipid rich core. Thin fibrous cap lines over the plaque separates it from the blood stream.

The activated macrophages and mast cells locally destabilize the plaque by secreting a variety of matrix – degrading proteolytic enzymes such as MMPs[124,125,126] and causes dissolution of fibrous cap resulting rupture of plaque.The exposed thrombogenic substances into the lumen leading thrombosis and its complications.

MMP-2s are again involved and can have ambivalent functions. The fibrous cap also contains elastin and proteoglycans[127,128]. Degradation of the ECM by MMPs can thin the fibrous cap and reduce the collagen content – typical features of vulnerable plaques prone to rupture[129.] MMPs also promote plaque angiogenesis, another feature associated with vulnerable plaques[130]. Facilitation of cell entry, destruction of ECM proteins, thinning of fibrous cap and angiogenesis mediated by MMP-2 crucial for the progression of plaque towards vulnerable, high-risk lesions. MMPs causes dissolution of fibrous cap and destabilization of atheromatous plaque resulting plaque rupture. Subsequently, exposes thrombogenic substance into the lumen resulting thrombosis and its complications.

Increased activation of MMP-2 in coronary plaques, associated with plaque clacification[131,132,133].. Besides local expression in

Fig 12.ROLE OF MMP-2 IN PLAQUE RUPTURE



plaques, circulating MMP-2 found in patients with myocardial infarction[134].

Additional evidence linking MMPs to plaque rupture is by cyclo oxygenase pathway. MMP-2 production by macrophage also occurs through a PGE2/cAMP- dependent pathway[135.]

MMP-2 AND PLATELET AGGREGATION

Translocation of MMP-2 from the cytosol to the platelet surface and is released during platelet aggregation[136]. vWF – induced GP Ib expression is mediated by MMP-2 and causes platelet adhesions[137]. The pro-aggregatory effects of collagen and platelets are initiated by MMP-2 via the mechanism independent of aspirin and thrombaxane[138]. It is thought to be beneficial that selective MMP-2 inhibitors could be given along with existing antiplatelet therapy in acute coronary syndrome.

ENDOGENOUS INHIBITORS OF MMP-2:

Activities of MMP-2 are inhibited by two types of endogenous inhibitors 1. α2-macroglobulin and 2.TIMPs . Human α2-macroglobin is a 725 kDa MW and it has four 180kDa identical subunits similar to MMP-2. It acts by entrapping the proteinase within the macroglobulin and the complex is easily cleared by the receptor -mediated endocytosis .

TIMPs, consisting of 184–194 amino acids , are subdivided into an N-terminal and a C-terminal sub domains. Three conserved disulfide bonds present in both domains, and the N-terminal domain fold act as an independent unit with MMP inhibitory activity.

MMP-2 GENE

MMP-2 gene is located at chromosome 16q21, size 27, 862 bases. Base pair starts from 55515474 and ends at 55540586 base pair. The DNA sequence contains 13 exons. . MMP-2 gene spans 17kb and contains 13 exons encoding a 72KDa protein, ~ 3.1kb of MMP-2 transcribed sequence, 1.9kb of promoter sequence, and ~ 1kb of intronic sequence.

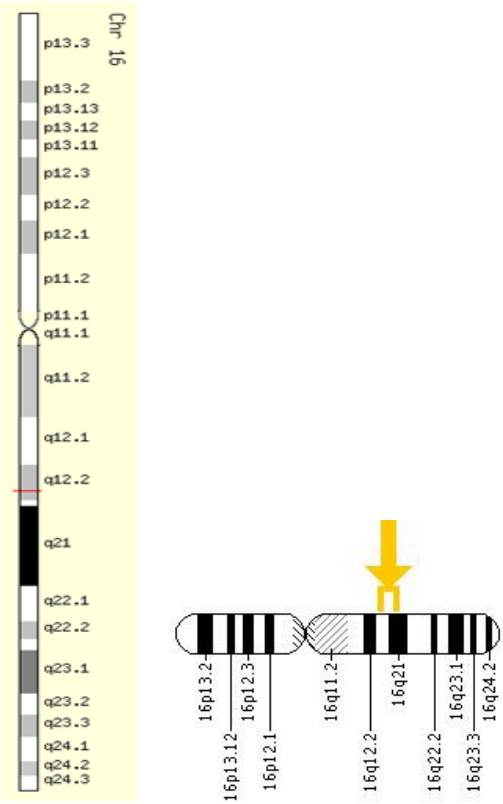
MMP-2 GENE POLYMORPHISM

MMP-2 gene polymorphism is situated at the promoter region linked with transcription. Price et al described this -1306 C/T MMP-2 polymorphism recently. They confirmed that C for T transition at location -1306 was functional and the C allele was linked with the more promoter activity.

It is a functional single nucleotide polymorphism[140]. -1306 C transition in the MMP-2 promoter sequence distrupts at SP1-type promoter site (CCACC box), and thus displays a strikingly lower promoter activity with the T allele. -1306 C > T polymorphism affect

Fig 13.MATRIX METALLOPROTEINASE-2 GENE

LOCUS 16q21



binding of the estrogen receptors and the SP1 transcription factor respectively[141]. In the MMP-2 gene [rs 243865: -1306C/T, and rs 2285053: -735C/T] there are two promoter region polymorphisms . The gene transcription and the enzymatic levels are altered by producing disruption of transcriptional regulators binding sites [142]. In the MMP-2 gene, the two C 735 T and C 1306 T are frequently present. They produce allele specific effects on the transcriptional activities of MMP gene promoters[143].

MMP-2 GENE POLYMORPHISM AND MMP-2 ACTIVITY.

Among individuals MMP-2 expression can vary due to genetic difference which could influence susceptibility to cardio vascular disease. Raised MMP-2 levels have been found in the plasma of MI patients[144]. Atherosclerotic plaques of coronary artery contain increased concentrations of MMP-2. In plasma MMP-2 concentration increase slowly after onset of MI, and reaches the maximum on day 21. The increased MMP-2 levels by the -1306CC genotype polymorphism becomes more significant and represents more MI risk[145].

POLYMORPHISM

The human genome contains hundreds of variations in base sequences that do not affect the phenotype .The property of molecules

to exist in more than one form is known as polymorphism. Polymorphism occurs in the frequency of more than 1% population.

MUTATION

A mutation is defined as an change in nucleotide sequence of DNA .This may be either gross ,so that large areas of chromosome are changed , or may be subtle with change in one or a few of every 10^6 cell divisions, one mutation takes place. Mutation may be defined as an abrupt spontaneous origin of new character. Statistically, out of every 10^6 cell divisions, one mutation takes place. Mutation occurs in the frequency of less than 1% of population.

DIFFERENCE BETWEEN MUTATION AND DNA POLYMORPHISM

If more than 1% of the population has a particular alteration in the sequence ,it is polymorphism. If only a few individuals have it, then it is mutation. Polymorphism is normal variation, and generally having no deleterious effect. Mutation is abnormal ,and sometimes will have defective function.

A polymorphic gene is one , in which the variant alleles are common in more than 1% of the total population. The existence of

two or more types of restriction fragment patterns is called restriction fragment length polymorphism (RFLP). This can be used as a genetic marker.

INHIBITORS OF MMP-2 EXPRESSION

In a concentration-dependent manner doxycycline inhibits expression of MMP-2 from smooth muscle cells. It reduces significantly MMP-2 production from SMCs at normal therapeutic serum concentration(5 μg/mL . It decreases half-life of mRNA from 49 hours to 28 hours, there by reducing MMP-2 mRNA stability. Doxycycline acts by binding to metal ions such as calcium and zinc and inhibits the MMP-2 expression.

OTHER DISEASES ASSOCIATED WITH MMP-2 GENE POLYMORPHISM.

Many polymorphisms in the promoter region of MMP-2 gene, affects the MMP-2 production in an allele-specific manner. All these functional polymorphisms are associated with the risk of nasopharyngeal carcinoma. There is notably raised susceptibility to NPC for the MMP2 -1306CC (rs243865:C>T) and -735CC (rs2285053:C>T) .The increased susceptibility to NPC related to the -1306CC and -735CC genotype and the C(-1306)-C(-735) haplotype was highly marked in heavy smokers. The *MMP-2* gene encoding 72-kDa

collagenase IV is located on chromosome 16q21. Recently, −735C>T (rs2285053), a sequence variant in the promoter region in *MMP-2*, leading to reduced promoter activity and thereby reduced gene expression, has been associated with gastric cardia . *MMP-2* −735C>T, CT or TT genotype recipients were linked with decreased danger for allograft rejection .MMP-2 members of the gelatinases subfamily, have been most widely associated with allograft rejection, suggesting a significantly increased gelatinase expression at the time of rejection. Development of tissue remodelling and fibrosis in the renal and liver allograft has been influenced by MMP-2 . By digestion of ECM ,MMPs initiate tumor development and metastasis in invasive cancers. MMP-2 easily degrades collagen IV and laminin-5 thereby supporting the metastatic cancerous cells to pass through by providing necessary gap .

In papillary thyroid microcarcinoma[146] expression of MMP-2 could be used as a prognostic marker.

Many reports showed that raised MP-2 expression and activity in pre cancer and cancer lesions of uterine cervix[147].

Progression of the lung diseases may also associated with elevated levels of MMP-2 were reported[148.] .

-1306 allele with more transcriptional activity was linked with high risk of cancers including lung , gastric , cardiac and colorectal cancer[149-152].

Over expression of MMP-2 has been reported in breast cancer[153]. Over expression has been reported in prostatic cancer[154]. Over expression has been reported in cutaneous cancer[155]. Elevated MMP-2 levels are linked with development of and MMP-2 activity in aneurysm of aorta[156].

Expression of COX2 and MMP-2 are linked with less survival in human breast cancer[157]. Increased MMP-2 levels have been reported after ischaemic stroke[158]. Beta-amyloid in neurons is degraded by active MMP-2 and play a role in Alzheimers disease[159]. Expression of MMP-2 In pancreatic duct adenocarcinoma[160] there will be increased expression of MMP-2 has been reported. Multicentric osteolysis and arthritis syndrome[161]results from mutation of MMP-2 gene.

Aim of the Study

AIM OF THE STUDY

Dysregulation in matrix metalloproteinase-2 activity plays a major role in atherogenesis and atherosclerotic plaque rupture, a disease characterised by difference in susceptibility among people in any given population. A strong positive relation exists between plasma matrix metalloproteinase-2 level and the risk of Myocardial Infarction.

Myocardial Infarction is predominantly due to atherosclerosis of coronary arteries . MMP-2 degrades extra- cellular proteins and plays an important role in atherogenesis and atherosclerotic plaque rupture, resulting thrombosis, and its complications like myocardial infarction. Available reports addressed on the variability of MMP-2 activity among people in given population. This variability is attributed to the various polymorphisms of MMP-2 gene, whose product is suspected to be involved in pathogenesis of atherogenesis and atherosclerotic plaque rupture with myocardial infarction.

The candidate gene of this study is MMP-2 gene and the aim of the study is, to determine the association of MMP-2 gene polymorphism and the concerned phenotype variation with Myocardial Infarction.

Materials and Methods

MATERIALS AND METHODS

STUDY POPULATION

CASES

The study sample comprised 100 unrelated Myocardial Infarction patients (85 male , 15 female) of Mean age 50.34 $\pm$ 9.84 years. More than 50% narrowing of at least one of the major coronary arteries included.. Hospitalized cases with acute attack of Myocardial Infarction were included.

CONTROL SUBJECTS

Controls were recruited from outpatient department during their visit for non cardiac cases. Age , Sex and other confounding factors like diabetes , hyper tension , smoking, alcoholism were matched.

METHODS

Recumbent blood pressure and 12 lead ECG were recorded. Height and weight were recorded and 5 mL of blood was collected by intravenous route after fortnight fasting in two test tubes. 2 mL was

heparanised and the remaining 3 mL was collected in EDTA tube. Heparanised tube was centrifuged at 2000 rpm for 10 minutes and Plasma was used for mmp-2 activity. EDTA tube was centrifuged at 2000 rpm for twenty minutes to get the buffy coat for DNA extraction and the plasma was utilized for lipid profile estimation.

BUFFY COAT SEPARATION

Buffy coat was separated by centrifugation of EDTA tubes at 2000 revolutions for 20 minutes. Buffy coat was transferred to 2mL eppendorf and was used for DNA extraction. Plasma separated was used for lipid profile estimation.

BIOCHEMICAL MARKERS

Total cholesterol (TC), high density lipoprotein cholesterol (HDL-c) .Low density lipoprotein cholesterol (LDL-c) and triglyceride concentration (TGL) Were determined by using enzymatic kits and XL-300 auto analyzer at Centralized Biochemistry Laboratory at R.G. G.G.H, Chennai-3.

DNA EXTRACTION BY MODIFIED HIGH SALT METHOD[161]

DNA EXTRACTION BY HIGH SALT METHOD

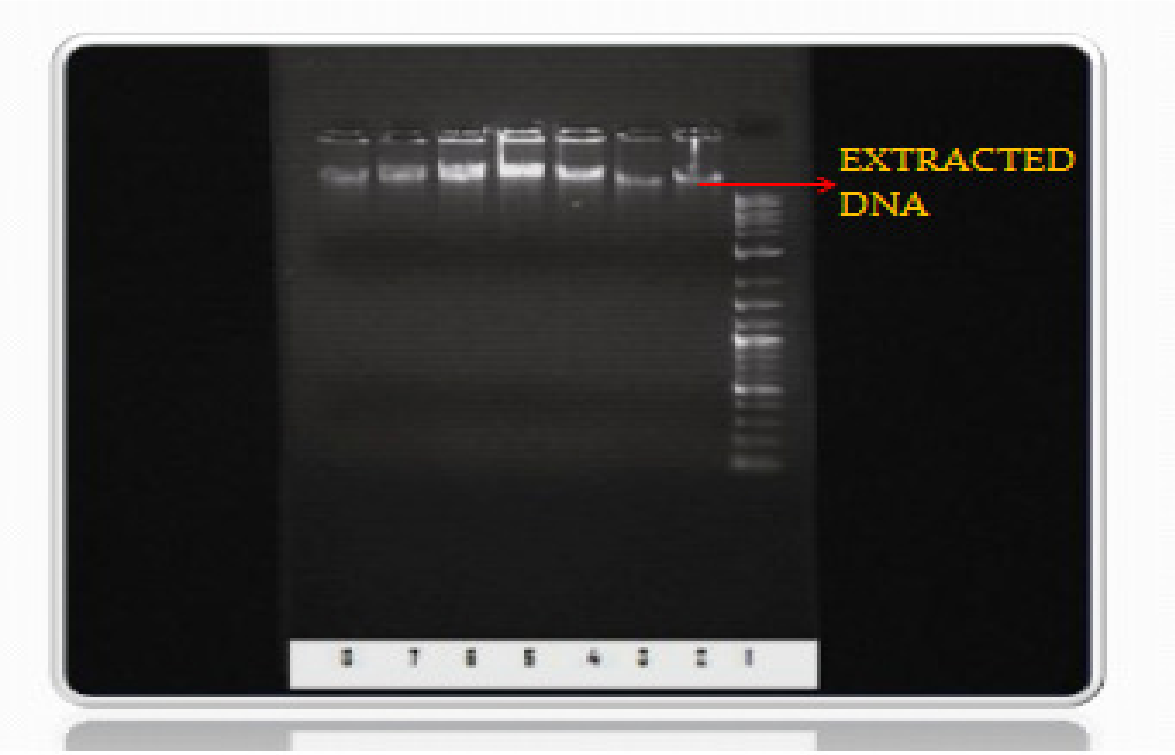


Figure 14. shows,extracted DNA(lane 2 to 8) was tested on 0.8% agarose gel using 1kbp ladder(lane 1)

Ladder shows 10000,8000,7000,6000,5000,4000,3000,2000,and 1000 bp fragments.

RBC Lysis:

- 400µL of buffy coat in a 2mL eppendorf is mixed with 1.6mL of 0.17M ammonium chloride and mixed by inversion until red cells are lysed for about 10 minutes.

- The cells are centrifuged at 4000 rpm for 10minutes.

- The white cell pellet is washed with 800µL of 0.17M ammonium chloride solution. The procedure is repeated till a clear white cell pellet is obtained.

WBC Lysis

- To the pellet 500 µL of TKM I solution is added. It is centrifuged at 10,000 rpm for 10 minutes.

Nuclear Lysis

- Discard the supernatant. To the pellet add 500 µL of TKM II solution. To that add 300 µL of 6M Nacl and 50 µL of 10% SDS.

- Mix well (vortex), Centrifuge at 10,000 rpm for 10 minutes.

- Save the supernatant. Transfer it to 1.5 Ml eppendorf.

DNA Precipitation

- To the supernatant double the volume of 100% ethanol is added.
- The sample is stored at -20◦C for 1 hour.
- Then it is centrifuged at 10,000 rpm for 20minutes at 4◦C in a refrigerated centrifuge.
- The supernatant is discarded. To this 500 µL of 70% ethanol is added. The pellet is mixed and centrifuged at 10,000 rpm for 10 minutes at 4◦C.
- Supernatant is discarded and the pellet is air dried.

Storage

- To the pellet 30 µL of LTE buffer is added and the extracted DNA is stored at -20◦C for future use.

Identification

- Extracted DNA was identified by 0.8% agarose gel electrophoresis with a constant voltage of 7V/ cm and comparison with a known molecular weight 1kb DNA ladder . Figure:1

Concentration of extracted DNA

- Concentration of extracted DNA was estimated using UV spectroscopy at 260nm. The absorbance at 260nm was 0.0203.

Concentration was calculated using the formula: 1 OD is equivalent to 50µg/mL

Conc. of DNA = absorbance × 50µg/mL × dilution factor

= 0.0203 × 50 × 100

= 101.5 ng / µL

- Purity of extracted DNA was assessed by 260/280 ratio and it was found to be > 1.7

POLYMERASE CHAIN REACTION

- 188 bp fragment of MMP-2 gene was polymerized by using,
    - Forward primer –5 CTTCCTAGGCTGGTCCTTACTGA 3
    - Reverse primer - 5 CTGAGACCTGAAGAGCTAAAGAGCT 3

Primer Reconstitution

Primers are supplied in lyophilized form. Autoclaved distilled water is used to prepare 100 × concentrations i.e. 10times the molecular weight of primer is the volume of water required to prepare 100 × concentrations which is 100 µmolar solution.

- From this stock solution 10 × concentration is prepared as the working solution for PCR.

POLYMERASE CHAIN REACTION

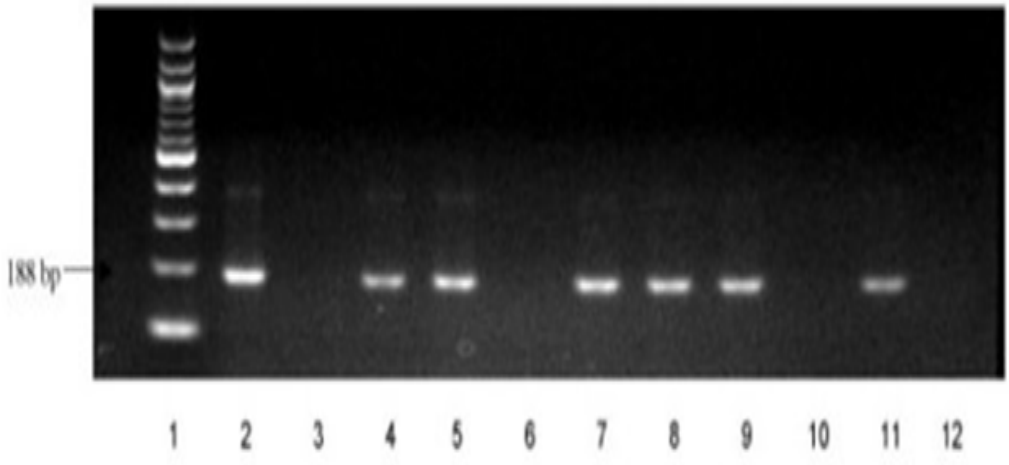


Figure15,shows,the188bpMMP2genePCRproduct(lane2to8)on2%agarosegel.

Lane1 shows,100bpDNAladder-markerfragmentsinclude1000,900,800,700,600,500,400,300,200,100bp.

MASTER MIX:

- Genei Red Dye master mix in the following composition was used.
- Master Mix consists of a unique inert red dye in addition to basic components necessary for PCR.
- Reaction buffer consisted of Tris Hcl - 10mM at pH 8.3
- KCl2 -50mM
- MgCl2 - 1.5mM acts as catalyst.
- dNTP's were used in a concentration of 2.5 mM each.
- Taq polymerase in a concentration of 1.5 U.
- Primers were used in a concentration of 5 pmol and DNA was used in a concentration of 200ng.
- PCR was done with a reaction volume of 25 µL with the following components;
- PCR master mix – 12.5 µL
- Forward primer – 0.8 µL

- Reverse primer – 0.8 µL
- DNA – 2 .0µL
- Distilled water – 8.9 µL
- Total – 25 µL

- Amplification was carried out in an Applied Biosystems thermal cycler with the following cycling conditions.

- Initial denaturation - 94^0 C -5min
- 37 cycles of
- Denaturation - 94^0C – 1 min
- Annealing - 60.5^0C – 1min
- Extension - 72^0C – 1min
- Final extension at 72^0C - 10 min.

- Amplified product – amplicons of 188 bp was identified by 2% agarose gel electrophoresis by comparison with a known 100bp DNA ladder. Figure 2.

AGAROSE GEL ELECTROPHORESIS

- PCR product is run on 2% agarose gel in a 30 mL agarose cast as follows: 0.75g of agarose is weighed and dissolved in 30mL of TAE buffer with a pH of 8.0.

- It is microwaved for 60 secs, cooled and 1.5 µL of ethidium bromide (10mg/mL) is added. It is poured into a cast and allowed to solidify for 15 min before it is kept in the electrophoresis tank.

- 8 µL of PCR product is loaded on to wells and 4 µL of 100bp DNA ladder is loaded on to single well as a marker. It is electrophoresed at 8V/cm for 45min and visualized under UV illumination.

RESTRICTION DIGESTION OF PCR PRODUCTS

MMP-2 polymorphism was determined by amplification by PCR and digestion with the Bfa1 restriction enzyme (7.5 units for 4 hours) followed by size fractionation in 3% Ethidium bromide –stained Agarose Gel Electrophoresis.

Principle of Bfa1(Bacteroides fragilis) enzyme digestion

C allele does not have the restriction site hence will yield a 188 bp fragment.

- T allele has the restriction site, hence gets cleaved to give 162 bp and 26 bp fragments.

- Heterozygous individuals (CT) allele gets cleaved to give 188bp,162bp,and 26bp fragments.

FIG:16,RESTRICTION DIGESTION PRODUCTS

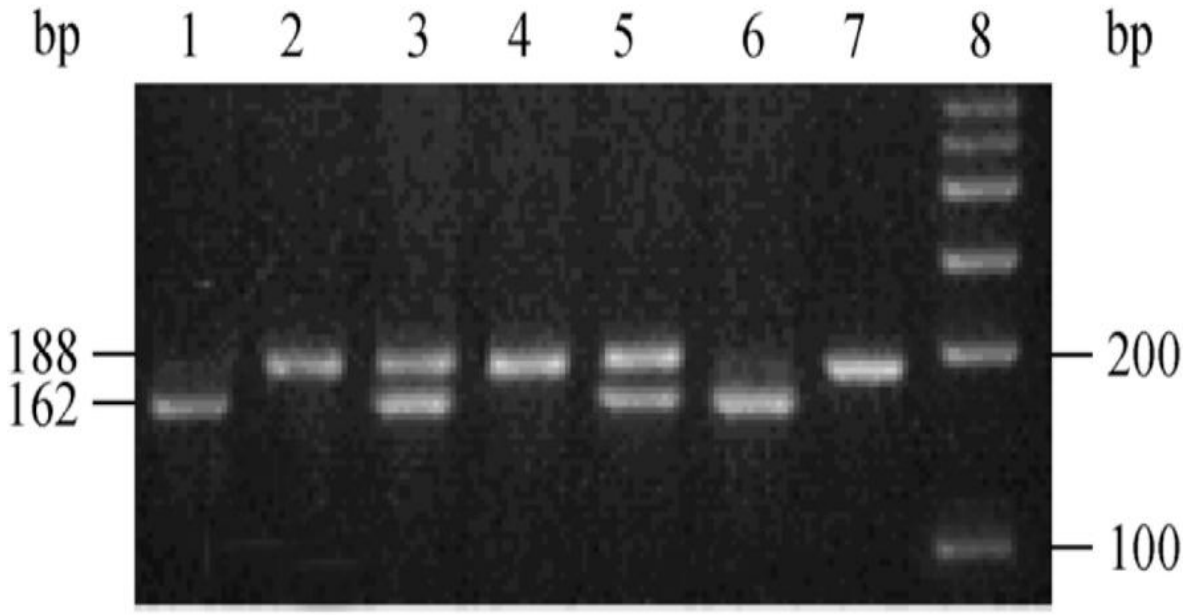


Fig 16,shows,genotype analysis done on 2%agarose gel electrophoresis using 100bp DNA ladder(lane1)

Lane8-Ladder

Lane(1,6)-TT

Lane(2,4,7)-CC,Lane(3,5)-CT

LIPID PROFILE

The biochemical parameters undertaken for the study were determined using the following methodologies:

Estimation of Plasma Total Cholesterol

Method

Cholesterol Esterase – Cholesterol Oxidase

Kit used

Liquixx of ERBA diagnostics Manheim GmbH Ltd.

Principle

$$\text{Cholesterol Ester} + H_2O \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4- Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Red quinine} + 4H_2O$$

Intensity of the red complex is directly proportional to the concentration of cholesterol which is measured at 505nm.

Reagents

Goods buffer (pH-6.4) 100mmol/L

Cholesterol Esterase	≥ 200U/L
Cholesterol Oxidase	≥ 100U/L
Peroxidase	≥ 3000 U/L
4- Aminoantipyrine	0.3 mmol/L
Phenol	5mmol/L

Standard (Cholesterol 200mg/dL)

Cholesterol	2g/L

Procedure

To 1 mL of the reconstituted reagent, 10 µL of plasma is added and reading is taken after 5 mins of incubation at 37^{o} C.

Reference Values

Cholesterol : 150-260 mg /dL

Estimation of Plasma Triglyceride

Method

Enzymatic Colorimetric method

Kit Used

Liquixx of ERBA diagnostics Manheim GmbH Ltd.

Principle

$$\text{Triglyceride} + H_2O \xrightarrow{\text{Lipoprotein Lipase}} \text{Glycerol} + \text{Fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol Kinase}} \text{Glycerol -3- Phosphate} + \text{ADP}$$

$$\text{Glycerol -3- Phosphate} \xrightarrow{\text{Glycerol -3- Phosphate Oxidase}} \text{Dihydroxy acetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4 Aminoantipyrine+3,5-DHBS} \xrightarrow{\text{Peroxidase}} \text{Red Quinone} + 2\ H_2O$$

The intensity of purple coloured complex formed during the reaction is directly proportional to the triglyceride levels.

Reagents composition.

Pipes buffer (pH -7.0)	40mmol/L
Lipoprotein Lipase	4000U/L

Glycerol Kinase	1500U/L
Glycerol -3- Phosphate Oxidase	4000 U/L
Peroxidase	2200 U/L
4- Aminoantipyrine	0.4 mmol/L
ATP	2 mmol/L
Magnesium	2.5mmol/L
DHBS(3,5-Dichloro -2 hydroxy Benzene sulphonate)	0.2mmol/L

Standard (Triglycerides 200mg / dL)

Glycerol (Trig.Equivalent)	2g/L

Procedure

To 1 mL of the reconstituted reagent 10 µL of plasma is added and read at 546nm after incubation at 37^{o}C for 5mins.

Reference Range

Males	60- 165 mg/dL
Females	40- 140 mg/dL

Estimation of HDL Cholesterol

Method Modified polyvinyl sulphonic acid (PVS) and polyethylene –glycol-methyl ether (PEGME)coupled classic precipitation method.

Kit used Erba XL System Pack

Principle

LDL,VLDL and chylomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL,VLDL and chylomicron (CM) by cholesterol oxidase (CHOD) and cholesterol esterase (CHER). The enzymes selectively react with HDL to produce hydrogen peroxide which, yields blue coloured complex upon oxidase condensation with TODB (N , N –Bis (4 sulphonyl)-3 methylaniline)and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD). The intensity of chromogen (Quinone) formed during the reaction directly is proportional to the HDL-C in the sample and is measured at 593nm.

$$\text{HDL} + \text{LDL} + \text{VLDL} + \text{CM} \xrightarrow[\text{PVS/PEGME}]{\text{PVS,PEGME}} \text{HDL} + (\text{LDL}+\text{VLDL}+\text{CM}).$$

CHER& CHOD

$$HDL\text{-}C + H_2O + O_2 \longrightarrow 4\text{-cholestenone} + \text{Fatty acid} + H_2O_2$$

Peroxidase

$$2H_2O_2 + TODB + 4AA \longrightarrow \text{Blue colored complex} + 2H_2O$$

Reagents composition

Reagent 1

MES buffer pH 6.5	6.5mmol/L
TODB(N,N–Bis(4sulphonyl)-3methylaniline)	3mmol/L
polyvinyl sulphonic acid (PVS)	50mg/L
polyethylene –glycol-methyl ether (PEGME)	30ml/L
Magnesium chloride (MgCl2)	2mmol
EDTA	
Detergent	

Reagent 2

MES buffer, pH – 6.5 50mmol/L

Cholesterol oxidase -20Ku/L

cholesterol esterase-5Ku/L

Peroxidase-5kU/L

4-Aminoantipyrine-0.9g/L

Detergent -0.5%

Calibrator

HDL-C 60mg/dL

Procedure

Reagent 1 & 2 are placed in the auto analyser with the following assay parameters:

Assay type	:	2 point
Primary wavelength nm	:	600,
Secondary wavelength nm	:	700
R-1 volume	:	270,
R-2 volume	:	70
Reaction direction	:	increasing,
Sample volume	:	3 µL
Calibration	:	straight

Reference Values

Adult male : 35.3 – 79.5 mg /dL

Adult female : 42.0 – 88.0 mg / dL

Estimation of LDL Cholesterol.

Method Modified polyvinyl sulphonic acid (PVS) and polyethylene – glycol-methyl ether (PEGME) coupled classic precipitation method.

Kit used

Erba XL System Packs

Principle

LDL,VLDL and chylomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL,VLDL and chylomicron (CM) by cholesterol oxidase(CHOD) and cholesterol esterase (CHER), whereas HDL reacts with the enzymes. Addition of reagent 2 containing a specific detergent releases LDL from the PVS/PEGME complex. The released LDL reacts with the enzymes enzymes to produce hydrogen peroxide which, yields blue coloured complex upon oxidase condensation with TODB (N , N –Bis (4 sulphonyl)-3 methylaniline)and 4- aminoantipyrine (4-AA) in the presence of peroxidase (POD). The intensity of chromogen (Quinone) formed during the reaction directly is proportional to the HDL-C in the sample and is measured at 593nm.

PVS,PEGME

HDL +LDL+ VLDL + CM ⟶ HDL+(LDL+VLDL+CM).
PVS/PEGME

CHER& CHOD

HDL-C + H_2O +O_2 ⟶ 4-cholestenone+ Fatty acid + H_2O_2

(LDL+VLDL+CM).PVS/PEGME+Detergent ⟶ LDL+(VLDL+CM).

PVS/PEGME .

CHER& CHOD

LDL+H2O+O2 ⟶ Cholest-4en -3 –one+Fattyacid+H2O2.

Peroxidase

2H2O2+4AA+TODB ⟶ Quinone+5H2O

Reagents composition

Reagent 1

MES buffer pH 6.5	50mmol/L
polyvinyl sulphonic acid (PVS)	50mg/L
polyethylene –glycol-methyl ether (PEGME)	30ml/L
Magnesium chloride (MgCl2)	2mmol
EDTA	
Detergent	
TODB (N,N-Bis(4-sulfobutyl)-3-methylaniline)	3mmol

Reagent 2

MES buffer pH 6.5 50mmol/L

EDTA

Detergent

TODB(N , N –Bis (4 sulphonyl)-3 methylaniline) 3mmol/L

Calibrator

LDL-C 107**mg/dL**

Procedure

Reagent 1 & 2 are placed in the auto analyser with the following assay

parameters:

Assay type	:	2 point
Primary wavelength nm	:	600,
Secondary wavelength nm	:	700
R-1 volume	:	210,
R-2 volume	:	70
Reaction direction	:	increasing,
Sample volume	:	3 µL

Calibration : straight

Expected values

Optimal <100mg/Dl

Near/above optimal - 100-129mg/dL

Boder line high - 130-159mg/dL

High -160-189mg/dL

Very high - 189mg/dL

VLDL Cholesterol

This parameter was calculated by using formula given below:

VLDL-C = TGL/5

Estimation of Plasma MMP-2 activity

MMP-2 activity in plasma was measured by ELISA

Principle

Heparinised plasma is used for measurement MMP-2 concentration by ELISA. The kit contains 96 – wells plate. Wells are coated with anti-human MMP-2 antibody . MMP-2 present in a sample is bound to the wells by immobilized antibody.Then biotinylated anti-human MMP-2

antibody is added. After washing away unbound biotinylated antibody , add HRP-conjugated streptavidin to the wells is added. The wells are again washed ,and TMB substrate solution is added and color develops in proportion to the amount of MMP-2 bound. The color changes from blue to yellow by the addition of stop solution and the intensity of the color is read at 450nm.

Reagents

1. MMP-2 Microplate coated with anti –human MMP-2.
2. Wash Buffer Concentrate.
3. Standards: Recombinant human MMP-2.
4. Assay Diluent: For Standard/Sample dilution.
5. Detection Antibody MMP-2: Biotinylated anti-human MMP-2.
6. HRP- Streptavidin Concentrate.
7. TMB One- Step Substrate Reagent.
8. Stop Solution.

Assay Procedure

Prepare all reagents samples, controls, and standards and bring them to room temperature before use.

1. Add 100µl standards ,samples and controls to each well. Incubate 2 hours at room temperature . Discard and wash.

2. Add 100 µl prepared biotin antibody to each well. Incubate 1 hour at room temperature. Discard the solution and wash each well with wash buffer.

3. Add 100 µl prepared HRP- Streptovidin solution. Incubate 45 minutes at room temperature. . Discard the solution and wash each well with wash buffer.

4. . Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.

5. Add 50 µl Stop Solution to each well. Read at 450nm immediately.

CALCULATION

Calculate the mean absorbance for each set of standards, samples and controls and substract the average zero standard optical density. Plot the standard curve on log-log graph paper with standard concentration on the x –axis and absorbance on the y-axis and draw the best-fit straight line through the standard points.

Statistical Analysis

STATISTICAL ANALYSIS

1. Allele frequencies were calculated by allele counting.

2. Age , BMI, serum lipid levels were compared between control subjects and cases by students t test.

3. Genotype frequency distribution between cases and controls were compared with a χ^2 test .

4. MMP-2 activity was compared between control and cases by Student t test $p<0.05$ was considered significant.

5. MMP-2 activity for both cases and controls was entered into a Microsoft Excel Spread Sheet. MMP-2 activity is compared between MMP-2 genotypes by using students t test.

6. Logistic regression analysis was performed to evaluate the interaction between human MMP-2 genotypes and other variables in relation to the prevalence of Myocardial Infarction. Independent variables included in the analysis were age (quantitative), sex (male/female),smoking(yes/no),Alcoholism(Yes/No),
   Hypertension (Yes/No), Diabetes (Yes/No), Serum Levels of Cholesterol , Triglycerides (Quantitative).

Results

MASTER CHART

Table-1: LIPID LEVELS, BMI AND GENOTYPE OF CASES

Sl No	AGE	SEX	Angio	DM	HYT	SMK	ALC	WT	HT	BMI	CHOL	TGL	HDL	LDL	MMP-2	MMP-2
1	48	M	SVD	NO	YES	YES	YES	66	1.57	26.78	159	194.7	26.8	92.3	747	CC
2	56	M	SVD	NO	NO	NO	YES	71	1.64	26.48	175.3	180.5	44.8	89.3	756	CC
3	49	F	SVD	NO	NO	NO	NO	63	1.57	25.68	175.7	175.6	44.7	90.4	816	CC
4	60	M	SVD	NO	YES	YES	YES	68	1.62	25.89	167.9	116.9	50.1	91.9	550	CT
5	53	M	SVD	YES	NO	NO	NO	59	1.52	25.77	196.7	194.5	24.4	90.4	534	CC
6	58	M	SVD	NO	NO	YES	NO	78	1.68	27.67	197.6	197.8	23.5	129.4	921	CC
7	57	M	SVD	NO	YES	YES	YES	58	1.64	21.67	174.7	96.8	59.4	130.5	782	CC
8	50	M	SVD	NO	NO	YES	YES	72	1.71	24.78	188.5	185.7	22.8	91.9	712	CC
9	55	F	TVD	YES	NO	NO	NO	74	1.57	29.98	214	195.7	40.4	124.6	823	CC
10	61	M	TVD	YES	YES	NO	NO	81	1.59	31.99	161.8	180.8	44.1	130.5	535	CT
11	47	M	SVD	NO	NO	YES	NO	58	1.64	21.57	158.8	184.8	40.3	77.5	861	CC
12	63	F	SVD	YES	NO	NO	NO	64	1.7	21.99	215.5	193.6	38.3	77.5	956	CC

13	55	M	SVD	YES	YES	YES	YES	71	1.62	26.87	210.2	198.9	31.9	134.5	400	TT
14	56	M	SVD	YES	YES	YES	YES	61	1.7	21.24	146.2	88.8	47	134.5	842	CC
15	58	M	SVD	NO	NO	NO	YES	56	1.61	21.78	207.7	182.5	43	84.9	922	CC
16	57	M	TVD	NO	NO	YES	NO	80	1.58	32.01	203.7	196.9	44.8	130.4	953	CC
17	48	M	DVD	NO	NO	YES	NO	62	1.69	21.78	160.5	188.4	44.7	130.4	717	CC
18	62	M	SVD	YES	YES	NO	NO	71	1.54	29.87	150.4	98.5	50.1	80.3	413	TT
19	54	F	SVD	NO	YES	NO	NO	57	1.64	21.24	176.9	98.4	24.4	80.2	545	CC
20	48	M	DVD	NO	NO	YES	NO	59	1.72	20.01	209.8	189.4	26.8	95.8	405	TT
21	49	M	DVD	YES	YES	YES	NO	79	1.71	27.02	168.1	106.5	59.4	130.5	782	CC
22	47	F	TVD	NO	NO	NO	NO	54	1.53	22.87	168.7	188.4	22.8	90.2	532	CT
23	59	F	SVD	YES	YES	NO	NO	83	1.64	30.88	174.6	196.9	40.4	90.1	832	CC
24	55	M	SVD	NO	NO	YES	YES	60	1.63	22.66	176.6	189.5	44.1	95.8	817	CC
25	54	M	DVD	NO	YES	NO	NO	62	1.61	23.98	179.7	87.3	40.3	100.5	523	CT
26	52	M	TVD	YES	NO	YES	YES	72	1.71	24.56	160.3	197.8	38.3	94.5	727	CC
27	60	M	DVD	NO	NO	YES	YES	69	1.67	24.78	206.3	80.6	31.9	90.2	754	CC
28	55	F	DVD	YES	YES	NO	NO	61	1.6	23.67	216.4	185.7	39.5	132.8	876	CC
29	52	M	TVD	YES	NO	YES	NO	69	1.71	23.68	172.2	90.2	36.8	129.5	417	CC
30	65	M	DVD	NO	YES	NO	NO	70	1.65	25.77	170.3	84.6	29.9	94.5	515	CT
31	56	M	TVD	NO	YES	YES	YES	75	1.58	29.99	175.3	196.4	38.5	92.4	789	CC

32	49	F	DVD	YES	NO	NO	NO	73	1.62	27.68	162.2	186.7	46.4	100.3	987	CC
33	57	M	SVD	NO	NO	NO	NO	58	1.6	22.79	199.4	193.5	57.4	82.8	403	TT
34	53	M	SVD	NO	NO	YES	YES	61	1.58	24.57	184.8	83.6	37.4	124.9	747	CC
35	57	M	TVD	NO	YES	NO	YES	84	1.71	28.67	201.4	188.4	52.6	106.3	859	CC
36	56	M	TVD	NO	NO	YES	YES	76	1.75	24.67	175.9	197.8	36.7	129.5	408	TT
37	61	M	DVD	YES	NO	NO	NO	64	1.64	23.68	201.9	84.8	35.4	95.7	822	CC
38	56	M	SVD	YES	YES	YES	NO	55	1.6	21.55	213.9	184.6	34.2	109.6	510	CT
39	48	M	TVD	NO	NO	YES	YES	77	1.62	29.46	206.8	179.6	63.7	134.7	867	CC
40	59	M	SVD	NO	YES	NO	NO	60	1.57	24.47	205.2	190.8	26.5	132.8	921	CC
41	61	M	DVD	NO	YES	YES	YES	60	1.66	21.79	152.5	195.9	27	137.4	942	CC
42	51	M	TVD	NO	NO	YES	YES	67	1.61	25.89	166.5	78.5	31.8	80.9	765	CC
43	50	M	SVD	YES	YES	NO	NO	69	1.64	25.66	200.6	189.7	24.6	94.5	516	CC
44	55	M	DVD	NO	YES	YES	YES	71	1.63	26.89	178.8	130.6	51.9	120.3	511	CT
45	57	M	SVD	NO	NO	YES	YES	67	1.67	23.99	156.1	188.9	31.1	102.4	678	CC
46	49	M	SVD	NO	N0	YES	YES	68	1.59	26.79	211.2	96.8	49.2	82.8	987	CC
47	54	M	SVD	NO	NO	NO	NO	62	1.55	25.87	183.6	135.8	34.6	142.1	504	CC
48	60	F	SVD	NO	YES	NO	NO	60	1.56	24.57	249.4	188.3	49.9	116.3	765	CC
49	53	M	SVD	NO	YES	YES	YES	76	1.6	29.68	169.3	184.6	37.9	153.8	400	TT
50	59	M	SVD	YES	NO	YES	YES	66	1.67	23.55	194.6	184.7	25.6	105.7	529	CT

51	50	M	SVD	YES	NO	YES	NO	73	1.72	24.57	194.6	186.9	54.7	106.9	876	CC
52	57	M	DVD	YES	NO	YES	YES	57	1.63	21.46	209.6	195.4	22.7	134.7	854	CC
53	52	M	SVD	NO	NO	YES	NO	68	1.65	24.88	211.2	193.7	46.3	115.8	652	CC
54	54	M	SVD	NO	YES	YES	YES	74	1.67	26.43	169.3	74.7	31.8	100.3	712	CC
55	49	M	DVD	NO	NO	NO	NO	63	1.63	23.54	208.1	185.7	52.7	98.5	759	CC
56	58	M	DVD	NO	YES	YES	YES	79	1.69	27.71	151.8	154.7	50.1	143.6	720	CC
58	53	F	DVD	NO	YES	NO	NO	68	1.62	25.81	179.2	164.6	29.6	92.5	875	CC
57	47	F	SVD	YES	NO	NO	NO	62	1.66	22.54	150.7	189.4	39.2	100.2	852	CC
59	49	M	DVD	YES	YES	NO	NO	73	1.74	24.21	168.9	189.7	38.9	80.9	489	CC
60	54	M	DVD	N0	NO	NO	NO	81	1.64	29.98	241.9	184.8	48.1	84.2	786	CC
61	62	M	TVD	YES	NO	NO	NO	76	1.69	26.53	191.5	74.7	43.1	159.4	389	CC
62	48	M	TVD	YES	YES	NO	NO	73	1.72	24.72	167.8	194.6	59.1	120.3	542	CT
63	53	M	TVD	YES	NO	YES	YES	72	1.65	26 .54	208.7	190.7	31.1	92.4	865	CC
64	52	M	TVD	No	NO	YES	YES	78	1.71	26.84	167.7	89.6	33.4	133.9	879	CC
65	49	M	TVD	NO	NO	NO	NO	56	1.58	22.34	217.2	190.4	39.9	88.6	530	CT
66	51	M	DVD	NO	NO	YES	YES	74	1.67	26.65	172.8	184.3	60.1	142.1	786	CC
67	48	M	DVD	NO	YES	NO	NO	73	1.65	26.88	161.7	190.5	32.9	93.4	432	CC
68	60	M	DVD	NO	YES	YES	YES	68	1.57	27.74	209.9	83.9	38.7	89.3	400	TT
69	59	F	SVD	N	NO	NO	NO	73	1.57	29.71	222.2	196.8	48.2	132.6	723	CC

70	62	M	DVD	NO	NO	YES	YES	72	1.67	25.82	171.6	196.9	47.5	153.8	874	CC
71	57	M	TVD	NO	NO	NO	NO	80	1.64	29.81	183.4	177.5	49.1	94.6	543	CT
72	59	M	DVD	YES	YES	NO	NO	57	1.68	20.24	209.3	154	35.2	116.9	823	CC
73	58	M	SVD	NO	NO	YES	YES	80	1.68	28.53	208.5	197.6	24.6	130.1	789	CC
74	60	F	DVD	NO	YES	NO	NO	73	1.66	26.41	185.3	189.7	34.9	129.7	795	CC
75	52	M	DVD	NO	YES	NO	NO	57	1.63	21.58	189.3	151.4	23.9	110.4	789	CC
76	61	M	TVD	NO	YES	YES	YES	78	1.65	28.75	166.3	192.4	40.1	93.5	806	CC
77	51	M	SVD	NO	YES	YES	YES	75	1.64	28.02	212	198.9	38.7	92.8	531	CC
78	55	M	SVD	YES	NO	NO	NO	64	1.65	23.53	169.42	172.5	31.3	154.8	765	CC
79	54	M	DVD	NO	YES	NO	NO	69	1.67	24.65	253.2	175.3	58.7	112.5	550	CT
80	50	M	TVD	YES	YES	YES	YES	82	1.66	29.83	185.9	164.6	39.7	54.8	890	CC
81	62	M	DVD	YES	NO	NO	NO	74	1.6	28.93	136.3	196.4	56.5	89.3	432	CC
82	55	M	TVD	YES	YES	YES	YES	73	1.62	27.72	143	111.9	31.5	132.7	541	CT
28	49	F	SVD	YES	YES	NO	NO	78	1.62	29.83	212	141.6	40.5	131.4	765	CC
84	48	M	DVD	NO	NO	NO	NO	76	1.65	27.81	219.9	80.6	34.8	143.6	962	CC
85	57	M	SVD	NO	YES	YES	YES	74	1.64	27.51	156.9	180.5	36.4	100.2	413	TT
86	54	M	SVD	NO	YES	YES	NO	73	1.71	24.92	168.7	88.4	34.2	89.4	942	CC
87	58	M	TVD	NO	YES	NO	NO	57	1.66	20.61	231.7	187.8	34.8	159.4	535	CT
88	53	M	SVD	NO	NO	YES	YES	75	1.64	27.9	202.6	96.9	37.2	125.9	426	CC

89	60	M	SVD	NO	NO	YES	YES	80	1.65	29.51	123.5	185.3	31.4	62.9	805	CC
90	49	M	DVD	NO	NO	YES	YES	55	1.63	20.71	169.4	189.8	34.6	90.3	467	CC
91	55	M	TVD	NO	NO	YES	YES	73	1.63	27.61	138.4	154.7	39.8	56.3	890	CC
92	52	M	SVD	YES	YES	YES	YES	78	1.62	29.84	164.2	74.8	35.7	87.9	532	CT
93	53	M	SVD	NO	NO	YES	YES	52	1.55	21.51	212.7	191.2	38.7	135.8	621	CC
94	52	M	TVD	NO	NO	YES	YES	57	1.54	23.96	226.3	183.7	36.8	145.9	764	CC
95	49	M	SVD	YES	NO	YES	YES	62	1.65	22.65	167.1	96.3	42.1	92.4	874	CC
96	62	F	SVD	YES	NO	NO	NO	82	1.66	29.72	160.5	190.5	36.9	90.5	671	CC
97	55	M	TVD	NO	YES	NO	NO	69	1.64	25.83	169.7	199.2	32.8	88.6	715	CC
98	57	M	SVD	NO	YES	YES	YES	66	1.69	22.94	151.7	190.7	36.2	64.7	540	CT
99	60	M	SVD	YES	YES	NO	NO	70	1.62	26.83	153.9	193.2	41.1	80.49	835	CC
100	51	M	SVD	NO	NO	NO	NO	82	1.66	29.83	192.2	175.4	37.2	123.4	921	CC

S.NO	Age (yrs)	Sex	Angio Finding	DM	HYT	SMK	ALC	WT(kg)	HT(m)	BMI (kg/m)	CHOL (mg/dL)	TGL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	MMP2 Activity(ng/ml)	MMP2 Genotype
1	54	M	NA	NO	YES	NO	NO	64	1.62	24.46	126.78	102.4	45.3	57.2	550	CC
2	52	M	NA	NO	NO	YES	NO	62	1.68	21.89	176.12	144.6	29.6	50.8	432	CT
3	51	M	NA	YES	NO	YES	NO	66	1.63	24.8	171.28	133.4	46.9	56.9	515	CC
4	49	F	NA	NO	YES	NO	NO	70	1.72	23.68	172.72	153.6	43.8	52.3	367	CC
5	47	M	NA	NO	NO	NO	YES	72	1.67	25.67	159.16	145.8	44.8	58.9	389	CT
6	60	M	NA	NO	NO	YES	YES	64	1.72	21.57	173.96	157.8	47.5	54.5	534	CC
7	58	M	NA	YES	NO	YES	NO	74	1.72	24.89	150.66	146.8	37.5	101.9	542	CC
8	54	M	NA	YES	NO	NO	YES	63	1.67	22.46	134.86	156.8	29.5	109.5	421	CT
9	49	M	NA	NO	YES	YES	YES	69	1.63	25.86	158.52	152.6	58.4	45.7	649	CC
10	49	M	NA	NO	YES	YES	NO	74	1.66	26.9	170.74	159.7	43.7	53.8	402	TT
11	51	F	NA	YES	NO	NO	NO	70	1.64	25.9	126.8	153.5	28.6	102.5	519	CC
12	54	M	NA	NO	YES	NO	YES	59	1.58	23.68	141.68	100.4	30.5	103.2	487	CT
13	59	M	NA	NO	NO	NO	YES	76	1.64	28.14	166.04	103.2	37.8	51.1	545	CC
14	56	M	NA	YES	NO	YES	YES	67	1.69	23.44	165.76	120.3	59.7	57.8	406	TT
15	53	M	NA	YES	NO	YES	NO	80	1.61	30.89	181.66	144.3	36.8	112.4	615	CC
16	59	M	NA	NO	YES	NO	YES	79	1.7	27.21	175.46	156.8	37.8	103.9	421	CT
17	54	M	NA	NO	YES	YES	NO	58	1.68	20.57	156.6	135.4	39.9	106.9	511	CC
18	56	M	NA	NO	YES	NO	YES	65	1.63	24.6	181.04	90.7	32.4	58.9	532	CC
19	55	M	NA	NO	NO	YES	YES	66	1.6	25.79	168.8	153.5	26.8	67.3	433	CT
20	60	F	NA	YES	NO	NO	NO	70	1.71	23.91	171.44	155.7	53.5	55.3	567	CC
21	54	M	NA	YES	NO	YES	YES	68	1.7	23.47	170.04	108.7	37.6	100.4	462	CC
22	57	M	NA	YES	NO	NO	YES	69	1.67	24.86	152.18	148.4	34.5	59.4	453	CT
23	58	M	NA	NO	YES	YES	NO	65	1.72	21.91	117.9	124.5	37.9	52.9	740	CC
24	55	M	NA	NO	NO	NO	YES	64	1.71	21.87	180.74	115.7	59.1	114.7	491	CC
25	59	M	NA	NO	YES	NO	NO	71	1.73	23.6	166.32	102.5	36.1	100.1	482	CT
26	60	F	NA	NO	NO	NO	NO	60	1.66	21.67	174.12	98.5	38.9	104.3	473	CC
27	61	M	NA	YES	NO	YES	YES	63	1.69	21.97	184.88	125.6	37.3	99.3	400	TT
28	60	M	NA	YES	NO	NO	YES	74	1.63	27.89	129.6	141.6	59.5	104.9	476	CC
29	54	M	NA	NO	YES	YES	YES	69	1.73	22.96	130.12	158.9	37.8	103.6	453	CT
30	51	M	NA	NO	YES	YES	YES	70	1.66	25.26	128.02	116.5	36.9	104.9	432	CC
31	53	F	NA	NO	NO	NO	NO	72	1.65	26.49	153.5	132.6	49	114.6	465	CC
32	56	M	NA	YES	NO	YES	YES	74	1.69	25.79	179.9	153.6	47.9	54.4	485	CC
33	59	M	NA	NO	YES	NO	NO	73	1.65	26.68	179.14	121.5	40.9	58.7	687	CT
34	57	M	NA	YES	NO	YES	YES	81	1.61	31.14	160.88	82.5	39.9	53.4	510	CC
35	62	M	NA	YES	NO	YES	YES	69	1.7	23.94	170.66	163.7	40.8	84.4	543	TT
36	57	M	NA	NO	YES	YES	NO	60	1.72	20.35	185.94	91.9	53.6	105.8	517	CC
37	55	M	NA	NO	YES	NO	YES	73	1.59	28.97	133.4	127.8	36.8	105.6	508	CT
38	49	M	NA	NO	NO	YES	YES	80	1.59	31.78	193.36	100.7	40.6	97.9	542	CC
39	48	M	NA	NO	YES	NO	NO	79	1.62	29.95	182.02	92.5	34.6	106.5	403	TT
40	50	M	NA	YES	NO	NO	YES	75	1.71	25.69	166.12	87.8	53.1	108.7	567	CC
41	55	M	NA	YES	NO	YES	YES	75	1.74	24.68	133.78	134.6	56.8	54.1	563	CC
42	56	M	NA	NO	YES	NO	YES	81	1.64	30.04	142.68	98.6	64.3	107	480	CT
43	59	M	NA	NO	YES	YES	NO	79	1.67	28.3	172.14	107.9	44.6	106	523	CC
44	51	M	NA	YES	NO	YES	NO	78	1.7	27.11	132.32	91.4	52.3	90.1	400	TT
45	58	M	NA	NO	YES	NO	YES	59	1.68	20.91	190.56	143.7	53.6	54.6	510	CC
46	52	F	NA	NO	NO	NO	NO	68	1.7	23.37	168.92	144.6	61.8	58.6	506	CT
47	57	M	NA	NO	YES	YES	YES	70	1.68	24.68	127.1	156.8	40.6	98.8	531	CC
48	53	M	NA	YES	NO	NO	NO	71	1.66	25.79	175.98	132.6	44.9	54.5	548	CC
49	56	M	NA	NO	YES	YES	NO	79	1.58	31.68	135.22	100.5	37.6	117.6	387	CT
50	54	M	NA	NO	NO	NO	NO	61	1.67	21.89	139.12	125.4	41.8	96.6	375	CC
51	55	M	NA	YES	NO	YES	YES	59	1.72	20.04	126.08	113.6	54.4	48.6	354	TT
52	56	M	NA	YES	NO	YES	NO	68	1.69	23.78	172.92	98.6	56.5	90.4	742	CC
53	54	M	NA	YES	NO	NO	YES	70	1.61	26.79	181.7	90.4	57.6	50.9	321	CT
54	49	M	NA	YES	NO	YES	NO	72	1.72	24.47	131.58	143.6	58.6	56.8	362	CC
55	48	F	NA	NO	YES	NO	NO	78	1.64	28.91	177.12	142.8	51.8	52.2	305	TT
56	53	M	NA	NO	NO	YES	NO	71	1.69	24.78	144.42	143.6	39.8	100.4	520	CC
57	57	M	NA	YES	NO	YES	YES	79	1.62	30.13	123.56	94.5	59.4	112.4	532	CC
58	52	M	NA	YES	NO	NO	NO	66	1.69	23.17	169.58	169.9	39.9	50.4	433	CT
59	59	M	NA	NO	YES	YES	YES	59	1.66	21.47	167.52	132.6	56.3	58.9	532	CC
60	51	M	NA	NO	NO	NO	NO	58	1.62	21.97	182.62	154.6	47.9	106.9	543	CC

61	59	M	NA	YES	NO	NO	YES	70	1.68	24.87	140.64	107.8	40.9	104.3	418	CT
62	61	F	NA	YES	NO	NO	NO	72	1.69	25.11	209.94	109.4	39.9	109.4	510	CC
63	48	M	NA	NO	YES	NO	YES	74	1.68	26.34	146.3	102.6	40.8	53.3	526	CC
64	55	M	NA	NO	NO	YES	YES	60	1.65	21.98	124.6	140.6	53.6	115.4	655	CT
65	56	M	NA	YES	NO	NO	NO	80	1.58	31.98	191.04	154.7	36.8	65.4	421	CC
66	52	M	NA	NO	YES	YES	YES	59	1.67	21.2	162.04	155.7	40.6	55.4	477	CT
67	58	M	NA	NO	NO	NO	NO	64	1.61	24.54	158.42	143.5	34.6	118.6	553	CC
68	56	F	NA	YES	NO	NO	NO	65	1.65	23.87	183.5	117	53.1	95.9	589	CC
69	54	M	NA	YES	NO	YES	YES	66	1.67	23.67	184.02	145.7	56.8	105	415	CT
70	51	M	NA	NO	YES	YES	NO	59	1.67	21.23	155.94	103.6	64.3	109	710	CC
71	49	M	NA	NO	NO	NO	YES	59	1.72	20.01	181.34	81.5	44.6	74	547	CC
72	58	M	NA	YES	NO	YES	NO	60	1.72	20.23	187.5	152.6	52.3	108	400	CT
73	57	F	NA	NO	YES	NO	NO	63	1.71	21.56	147.34	94.7	53.6	56.4	487	CC
74	60	M	NA	YES	NO	YES	NO	57	1.61	21.89	139.64	156.7	61.8	49.8	322	CT
75	53	F	NA	YES	NO	NO	NO	72	1.67	25.67	144.06	142.5	40.6	73.8	512	CC
76	52	M	NA	NO	NO	YES	YES	62	1.72	20.87	185.14	145.7	44.9	119	432	CT
77	48	M	NA	NO	YES	NO	NO	73	1.69	25.64	169.72	144.7	37.6	100.5	543	CC
78	60	M	NA	YES	NO	YES	YES	79	1.68	27.98	127.4	86.8	41.8	50.4	387	TT
79	56	M	NA	NO	NO	YES	NO	75	1.73	24.98	168.34	159.7	54.4	94.3	546	CC
80	59	M	NA	YES	NO	YES	NO	78	1.71	26.97	122.62	155.6	56.5	49.9	390	CT
81	51	F	NA	NO	YES	NO	NO	79	1.65	28.99	185.08	80.5	57.6	102	521	CC
82	58	M	NA	YES	NO	NO	NO	76	1.61	29.21	137.5	130.7	58.6	56.8	456	CT
83	52	M	NA	YES	YES	YES	YES	69	1.68	24.54	181.88	133.5	51.8	100.9	512	CC
84	57	M	NA	NO	NO	YES	NO	77	1.64	28.75	184.54	130.7	39.8	114	377	CT
85	53	M	NA	NO	NO	NO	NO	62	1.68	21.99	143.36	142.6	59.4	112	365	CC
86	56	M	NA	YES	NO	NO	NO	65	1.73	21.54	151.82	88.9	39.9	53	325	CC
87	55	F	NA	YES	NO	NO	NO	60	1.71	20.6	139.36	157.5	56.3	50.4	375	CT
88	54	M	NA	NO	YES	NO	NO	61	1.69	21.4	156.38	150.4	48	58.9	538	CC
89	49	M	NA	NO	YES	NO	NO	78	1.67	27.88	140.9	165.7	47.9	106.9	326	CT
90	44	M	NA	YES	NO	YES	NO	77	1.62	29.31	131.55	96.8	40.9	104.3	525	CC
91	60	M	NA	NO	YES	YES	NO	79	1.61	30.54	172.24	150.6	39.9	109.4	401	TT
92	54	M	NA	NO	NO	NO	NO	69	1.68	24.56	175.24	84.8	40.8	53.3	525	CC
93	48	M	NA	YES	NO	YES	NO	68	1.68	23.99	145.72	90.4	53.6	115.4	401	CT
94	61	M	NA	NO	YES	NO	YES	66	1.64	24.57	191.5	92.5	36.8	65.4	461	CC
95	48	M	NA	YES	NO	YES	YES	65	1.73	21.79	136.68	157.7	40.6	55.4	300	CC
96	54	M	NA	YES	NO	NO	NO	60	1.62	22.76	142.44	114.7	34.6	118.6	500	CT
97	58	M	NA	NO	YES	NO	NO	71	1.73	23.67	135.02	149.7	53.1	95.9	543	CC
98	57	M	NA	NO	NO	YES	NO	69	1.66	24.98	126.52	142.6	56.8	105	526	CC
99	59	F	NA	YES	NO	NO	NO	74	1.66	26.78	137	150.5	64.3	109	523	CC
100	54	F	NA	YES	NO	NO	NO	76	1.65	27.87	139	150.4	44.6	74	402	TT

TABLE 3: CHARACTERISTICS OF PATIENTS WITH MI AND OF CONTROL SUBJECTS

Variables	Case	Control	P value
Age	55.0±8.8	55.0±7.6	0.273 –NS
Sex Male	85(85%)	85 (85%)	0.495 –NS
Female	15 (15%)	15 (15%)	0.495-NS
DM	34 (34%)	44(44%)	0.094–NS
HT	45(45%)	35 (35%)	0.149 –NS
SMK	54(54%)	48(48%)	0.991 -NS
ALC	46(46%)	42(42%)	0.565-NS
BMI	25.69±0.57	24.91±5.80	0.638-NS
CHOLESTEROL	184.73±5.05	158.47±4.25	0.001-S
TRIGLYCERIDES	162.01±8.40	128.45±5.04	0.001-S
HIGH DENSITY LIPOPROTEIN	39.21±1.90	45.98±1.84	0.004-S
LOW DENSITY LIPOPROTEIN	108.25±4.77	83.29±4.97	0.001-S

Fig 17,GENOTYPE DISTRIBUTION OF MMP-2 GENE

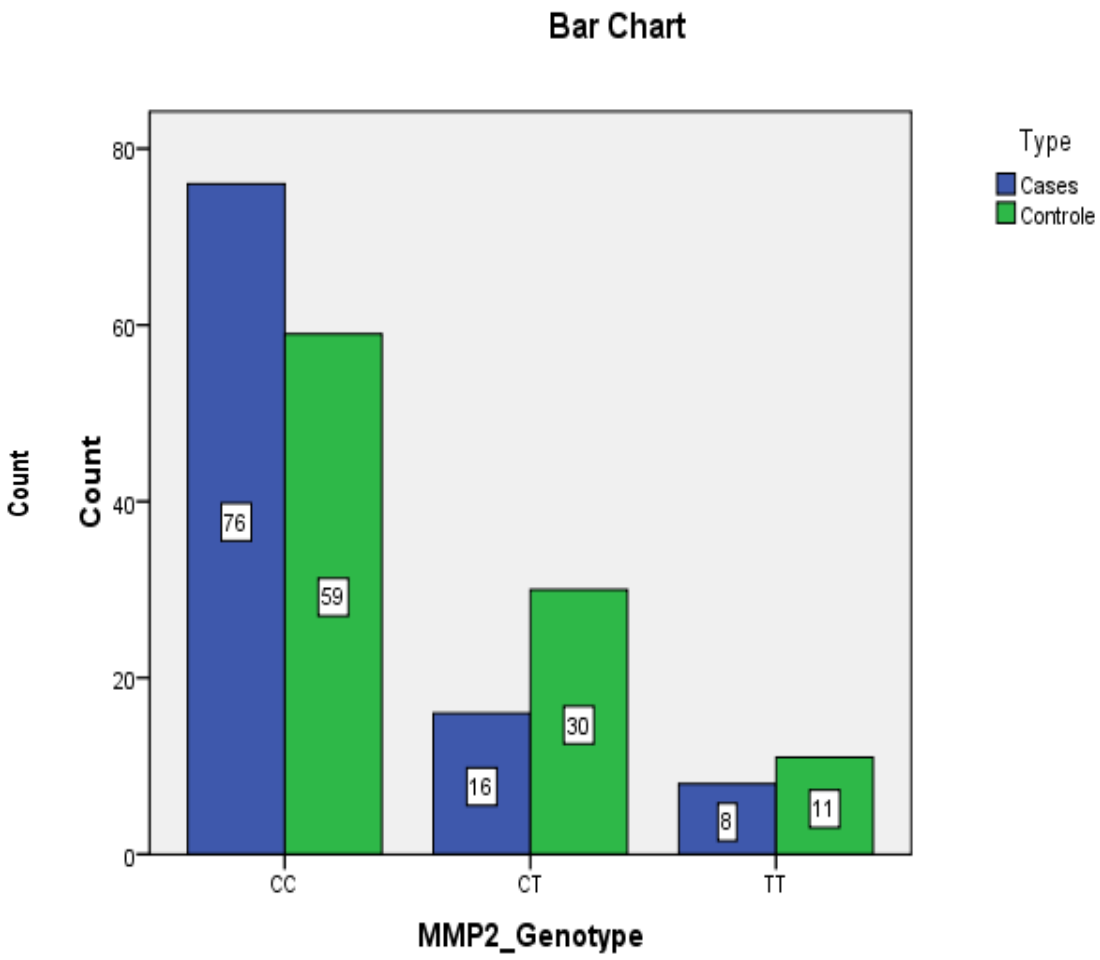


Table 4,GENOTYPE DISTRIBUTION OF MMP-2 GENE

			Type			P Value
			Cases	control	Total	
MMP2_Genotype	CC	Count	76	59	135	
		% within MMP2_Genotype	56.3%	43.7%	100.0%	
	CT	Count	16	30	46	Chi – sq. = 6.87
		% within MMP2_Genotype	34.8%	65.2%	100.0%	P = 0.032
	TT	Count	8	11	19	
		% within MMP2_Genotype	42.1%	57.9%	100.0%	
Total		Count	100	100	200	
		% within MMP2_Genotype	50.0%	50.0%	100.0%	

TABLE 5: COMPARISON OF MMP-2 ACTIVITY AMONG CASES AND CONTROLS

Variable	Case	Control	P value
ACTIVITY (ng/mL)	698.02 $\pm$178.05	481.35 $\pm$90.45	0.001 –S

Fig 18 ,COMPARISON OF MMP-2 ACTIVITY AMONG CASES AND CONTROLS

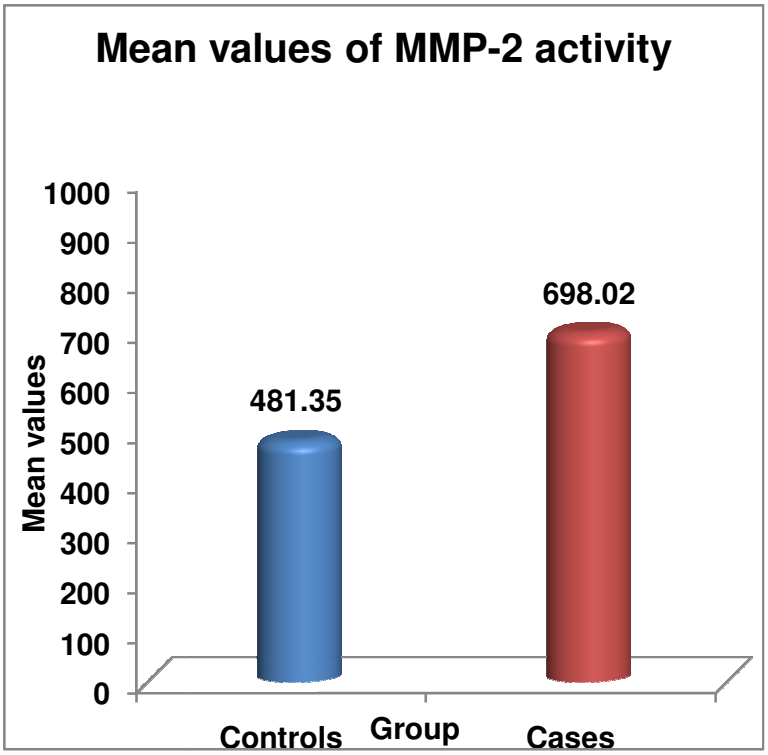


TABLE 6: RELATIONSHIP BETWEEN MMP-2 ACTIVITY AND GENOTYPE

Genotype	MMP-2 Activity(ng/ml)	P Value
CC	655±174	.0001-S
CT	472±78	.0001-S
TT	402±42	.0001-S

Fig 19, RELATIONSHIP BETWEEN MMP-2 ACTIVITY AND GENOTYPE

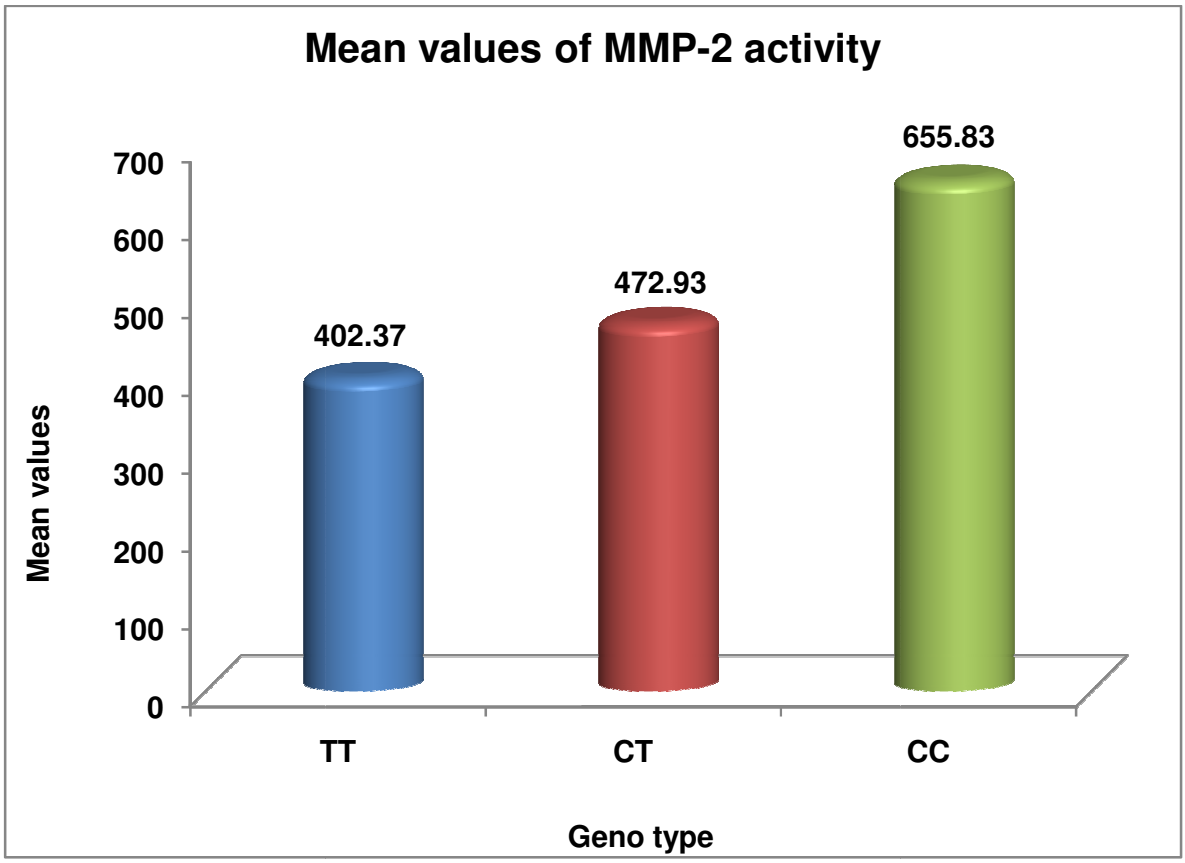


TABLE-7: MULTIPLE LOGISTIC REGRESSION ANALYSIS

Variables	B	S.E	WALD	df	sig	Exp(B)	UPPER	LOWER 95%
AGE	.081	.074	1.204	1	.273	1.084	.938	1.252
SEX	-.848	1.242	.466	1	.495	.428	.038	4.884
SMOKING	-.009	.776	.000	1	.991	.991	.217	4.538
ALCOHOLISM	-.402	.699	.331	1	.565	.669	.170	2.634
DIABETES	-1.246	.745	2.796	1	.094	.288	.067	1.239
BMI	.823	1.748	.222	1	.638	2.277	.074	70.002
TOTALCHOLESTEROL	.059	.017	11.948	1	.001	1.061	1.026	1.097
TRIGLYCERIDES	.041	.010	15.047	1	.000	1.041	1.020	1.063
HDL	-.105	.037	8.164	1	.004	.900	.838	.968

LDL	.062	.017	12.589	1	.000	1.064	1.028	1.101
MMP-2 ACTIVITY	.015	.003	21.993	1	.000	1.015	1.009	1.022
MMP-2 POLYMORPHISM CC	-.782	1.006	.604	1	.007	1.958	0.664	3.289
MMP-2 POLYMORPHISM CT	-.640	.986	.421	1	.009	1.527	0.576	3.543
INTERCEPT	-.625	92.567	.132	1	.716			

a. The reference category is: Control

RESULTS

- Table 3 shows Age , Sex , BMI , High Density Lipoprotein levels and conventional risk factor distribution among cases and control subjects. We obtained a nonsignificant p value with respect to all the confounding variables like age, sex, BMI, history of diabetes, hypertension, smoking, alcoholism. There was a significant difference in Total cholesterol level (high in cases 184.73±5.05), low in controls 158.47±4.25), Triglycerides level (high in cases 162.01±8.40),low in controls128.45±5.04), High Density Lipoprotein level (low in cases 39.21 +1.90),high in controls 45.98±1.84), Low Density Lipoprotein level (high in cases 108.25±4.77), low in controls (83.29±4.97).

- Table 4 shows Genotype distribution of human matrix metallo proteinase-2 gene in patients with MI and control subjects.

- Table 4 shows CC genotype was more frequent among cases (76%) when compared to controls (59%) . In contrast TT was more common among controls (11%) when compared to cases(8%). Distribution of CT genotype also between cases (16%) and controls (30%). P value is 0.032. The Allele frequencies were CC=135, CT=46, TT=19. This was found to be in Hardy Weinberg equilibrium.

- Table 5 shows the comparison of Matrix Metalloproteinase-2 activity among cases and controls. Significantly high MMP-2 activity

could be observed among cases (698±178) when compared to controls. (481 ± 90).Pvalue <0.001.

- Table 6 shows the difference in MMP-2 activity between CC,CT genotype and TT genotype. The activity was significantly higher among CC genotype individuals (655±174) when compared to CT(472± 78) and TT (402 ± 42) genotype individuals . Pvalue <0.001.

- Table 7 shows,Multiple logistic regression analysis. After correction of all confounding variables CC genotype remains significant between cases and controls.

Discussion

DISCUSSION

Genetic factors and various environmental factors are involved in a combination with development of MI. The susceptibility to MI is a complex trait[162] . This study was conducted to determine the association of MMP-2 polymorphism and its related MMP-2 activity with Myocardial Infarction. The three human MMP-2 genotypes and phenotypes were determined in 100 patients with MI confirmed by angiography and 100 control subjects.

The insignificant p value with respect to all the confounding variables like age ,sex, BMI, history of diabetes, hypertension ,smoking, alcoholism, showed that the cases and controls groups had been perfectly matched.The plasma MMP-2 levels are increased in cases when compared to controls. The significantly high MMP-2 levels in cases(698±178) ,controls(481±90)P(<0.001) re-emphasizes the fact that causes atherogenesis and plaque rupture.

When genotype analysis was performed , distribution of CC genotype was significantly higher among cases (76%) when compared to controls (59%). Pvalue was 0.032 showing that is significant. This indicates that CC genotype is an independent risk factor for atherosclerotic plaque rupture.The evidence available showed that there is a significantly high MMP-2 activity among cases (698±178) when compared to controls (481±90). P value was less than 0.001. This

shows that high MMP-2 activity is an independent risk factor for atherosclerosis.

When MMP-2 activity compared between MMP-2 genotypes there was a significantly high MMP-2 activity among CC genotypic individuals (655±174).and CT genotypic individuals (472±78), when compared to TT genotypic individuals (402±42). P value was (<0.001), suggesting the fact that CC genotype is associated with high activity and this high activity makes a person more susceptible to atherosclerosis. Hence CC genotype and the resultant high MMP-2 activity can be considered as an independent risk factor for atherosclerotic plaque rupture.

On multiple logistic regression analysis, CC genotype will increase the susceptibility of atherosclerosis. The odds ratio on multiple logistic regression analysis was 1.95 i.e., a person with CC genotype is 1.95 times at a risk for atherosclerosis after all the confounding variables are matched

Conclusion

CONCLUSION

- The high plasma MMP-2 activity and the CC genotype may be independent risk factors for Myocardial Infarction.

- MMP-2 activity can be used as a parameter for assessing Myocardial Infarction risk .

- MMP-2 activity can also be used to assess the outcome of atherosclerotic plaque rupture.

Future Prospects of the Study

SCOPE FOR FURTHER STUDY

- Research aimed at identifying the strategies to inhibit Matrix Metallo Proteinase-2 activity can be supported.

- Other Matrix Metallo Proteinase-2 gene polymorphisms may be explored to highlight their association with Matrix MetalloProteinase-2 activity and atherosclerosis and plaque rupture.

- Various transcriptional factors modulating the Matrix Metallo Proteinase-2 gene expression can be studied.

- MMP-2 inhibitors and TIMP-2 therapy can be tried in all those who have higher MMP-2 gene expression. But since atherosclerosis is a multifactorial disease, it does not look promising.

References

REFERENCES

1. Dodu SR. Coronary heart disease in developing countries: the threat can be averted. WHO Chron 1984; 38:3-7.

2. Akinkugbe OO. Epidemiology of cardiovascular disease in developing countries. J Hypertens 1990; 8 (Suppl.):S233-8.

3. Ciruzzi M., Schargrodsky H., Pramparo P., Rivas Estany E., Rodriguez Naude L., de la Noval Garcia R. et al. 2003 Attributable risks for acute myocardial infarction in four countries of Latin America. Medicina (Buenos Aires) 63, 697-703.

4. Newby A. C. 2005 Dual role of matrix metalloproteinases (matrixins) in intimal thickening and atherosclerotic plaque rupture. Physiol. Rev. 85, 1–31.

5. Tedgui A. and Mallat Z. 2006 Cytokines in atherosclerosis: pathogenic and regulatory pathways. Physiol. Rev. 86, 515-581.

6. Johnston JJ, Ginsberg SS, Vanwart HE, Berliner N (1992) "structure and expression of neutrophil gelatinase cDNA-Identify with type IV collagenase from HT 1080cells". J. Biol. Chem. 267(35): 25228-32. PMID 1460022

7. Entrez Gene: MMP2 matrix metallopeptidane 2 (gelatinase A, 72Kda gelatinase, 72Kda type IV collagenase) from HT 1080 cells. J. Biol. Chem. 267(35): 25228-32. PMID 1460022

8. Strongui AY, Collier IE, Bannitzov G, Marman BL, Grant GA & Goldberg G1 (1995) mechanism of cell surface activation of 72Kda type IV collagenase. Isolation of the activated form of the, membrane metalloproteinase. J. Biol Chem 270: 5331-5338.

9. Abilleira S., Bevan S. and Markus H. S. 2006 The role of genetic variants of matrix metalloproteinases in coronary and carotid atherosclerosis. J. Med. Genet. 43, 897-901.

10. Price S. J., Greaves D. R. and Watkins H. 2001 Identification of novel, functional genetic variants in the human matrix

metalloproteinase-2 gene: role of Spl in allele-specific transcriptional regulation. J. Biol. Chem. 276, 7549-7558.

11. Kai H., Ikeda H., Yasukawa H., Kai M., Seki Y., Kuwahara F. et al. 1998 Peripheral blood levels of matrix metalloproteases-2 and -9 are elevated in patients with acute coronary syndromes. J. Am. Coll. Cardiol. 32, 368-372.

12. Pasterkamp G., Schoneveld A. H., Hijnen D. J., de Kleijn D. P., Teepen H., van der Wal A. C. and Borst C. 2000 Atherosclerotic arterial remodeling and the localization of macrophages and matrix metalloproteases 1,2 and 9 in the human coronary artery. Atherosclerosis 150, 245-253.

13. Circulation. 1993 Mar: 87 (3 suppl) : 113-16. Coronary atherosclerosis. A multifactorial disease. Badimon JJ, Fuster V, Chesebro JH, Badimon L.

14. http://www.framinghamheartstudy. Org/risk/hrdcoronary.html

15. National Cholesterol Education Programme. Second report of the Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II. Bethesda, Md : National Heart Lung and Blood Institute, 1993. (NIH publication no. 93-3095)

16. Subendothelial Lipoprotein Retention as the Initiating Process in Atherosclerosi, Update and Therapeutic Implications. Ira Tabas, MD, PhD; Kevin Jon Williams, MD; Jan Boren, MD, PhD. DOI: 10.1161/CIRCULATIONAHA.106.676890.

17. Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4 444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 1994; 344: 1383-9.

18. Shepherd J, Cobbe SM, Ford I, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. N Engl J Med 1995; 333; 1301-7.

19. Ross R. The pathogenesis of atherosclerosis: A perspective for the 1990s. Nature 1993; 362: 801-809.

20. Stary HC, Chandler AB, Glasgov S, Guyton JR, Insull W Jr, Rosenfeld ME et al. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. Circulation 1994; 89: 2462-2478

21. Giovanni Davi, Carlo patron. Mechanisms of disease. Platelet activation and atherothrombosis. N ENGL J MED 2007; 357: 2482 – 2494.

22. Braunwald E. Shattuck Lecture – cardiovascular medicine at the turn of the millennium : triumphs, concerns, and opportunities. N Engl J Med 1997; 337:1360-9.

23. Long ER. The development of our knowledge of arteriosclerosis.In:Cowdry EV, ed.Arteriosclerosis:A Survey of the problem. New York: Macmillan; 1933:19-52.

24. Virchow R. Gesammelte Adhandlungen Zur Wissenschaftlichen Medicin. Frankfurt-am-Main: Meidinger Sohn; 1856:458-636

25. Stary HC. Macrophage foam cells, and eccentric intimal thickening in the coronary arteries of young children. Atherosclerosis 1987; 64: 91-108.

26. Anitschkow NN. A history of experimentation on arterial atherosclerosis in animals. In: Blumenthal HT, ed. Cowdry's Arteriosclerosis, 2d ed. Springfield, IL: Charles C Thomas; 1967:21-44.

27. Gimbrone MA Jr. Vascular endothelium in health and disease. In: Haber E, ed. Molecular cardiovascular Medicine. 1995: 387-399.

28. Dicorleto PE, Gimbrone MAJr. Vascular endothelium. In: Fuster V, Ross R, Topol EJ, eds. Atherosclerosis and coronary artery disease. Philadelphia: Lippincott- Raven; 1996: 387-399.

29. Griedling KK, Alexander RW. Endothelial control of the cardiovascular system: Recent advances. FASEB J 1996; 10:283-292.

30. Moncada S, Higgs EA, Vane JR. Human arterial and venous tissue generate prostacyclin (prostaglandin x), a potent inhibitor of platelet aggregation. Lancet 1977; 2: 18-20.

31. Synder SH. No endothelial NO. Nature 1995; 377: 196-197.

32. Stein Y, Stein O. Interaction between serum lipoproteins and cellular components of the arterial wall. Biochem Atheroscler 1979;7:313-344

33. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: The multistep paradigm. Cell 1994; 76: 201-314.

34. Carlos TM, Harlan JM. Leukocyte – endothelial adhesion molecules. Blood 1994; 84:2068-2101.

35. Bevilacqua MP. Endothelial – leukocyte adhesion molecules. Annu Rev Immunol 1993;11:767-804.

36. Hajjar KA, Hajjar DP, Silverstein RL, Nachman RL. Tumor necrosis factor mediated release of platelet derived growth factor from cultured endothelial cells. J Exp Med 1987; 166:235-245.

37. Wenger NK, Speroff L, Packard B. Cardiovascular Health and disease in women. Greenwich, CT: Lejacq communications; 1993.

38. McGovern PG, Pankow JS, Shahar E, Doliszny KM, Folssom AR, Blackbyrn H et al. Recent trends in acute coronary heart disease: Mortality, morbidity, medical care and risk factors. N Engl J Med 1996; 334: 884-890.

39. Muarbito JM, Evans JC, Larson MG, Levy D. Prognosis after the onset of coronary heart disease: An investigation of differences in outcome between the sexes according to initial coronary disease presentation. Circulation 1993; 88: 2548-2555.

40. Weintraub WS, Wenger NK, Jones EL, Craver JM, Guyton RA. Changing clinical characterisctics of coronary surgery patients: Differences between men and women; 1993; 88: (suppl II) 79-86

41. Chiriboga DE, Yarzcbski J, Goldberg JR, Chen Z, Gurwitz J, Gore JM, et al. A community wide prospective of gender differences and temporal trends in the use of diagnostic and revascularization procedures for acute myocardial infarction: Is there an evidence for gender bias? Ann Intern Med 1992; 116: 785-790.

42. Steingart RM, Packer M, Hamm P, Coglianese ME, Gersh B, Geltman EM, et al. Sex differences in the management of coronary artery disease. N Engl J Med 1991; 325: 226 – 230.

43. Krumholz HM, Douglas PS, Lauer MS, Pasternak RC. Selection of patients for coronary angiography and revascularization early after myocardial infarction: Is there an evidence for gender bias? Ann Intern Med 1992; 116: 785 – 790.

44. Funk M, Griffey KA. Relation of gender to the use of cardiac procedures in acute myocardial infarction. Am J Cardiol 1994; 74: 1170 – 1173.

45. Ayanian JZ, Epstein AM,. Differences in the use of procedures between women and men hospitalized for CAD. N Engl J Med 1991; 325: 221 – 225.

46. Williams JK. Shively CA, Clarkson TB. Determinants of coronary artery reactivity in premenopausal female monkeys with diet induced atherosclerosis. Circulation 1994; 90: 983-987.

47. Hopkins PN, Williams RR. Human genetics and coronary heart disease. A public health perspective. Annu Rev Nutr 1989; 9:303-345.

48. Expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult treatment panel II). Circulation 1994; 89: 1329 – 1445.

49. Rissanen AM. Familial occurrence of coronary heart disease: Effect of age at diagnosis. Am j Cardiol 1979; 44: 60-66.

50. Williams RR, Hopkins PN, Wu LL, Schumacher C, Hunt SC. Evaluating family history to prevent early onset coronary heart disease. In: Pearson TA, Criqui MH, Luepker RV, Oberman A, Winston M, eds. Primer in preventive cardiology. Dallas: American heart association; 1994: 93-106.

51. Kaplan GA, Keil JE. Socioeconomic factors and cardiovascular disease. A review of literature. Circulation 1993; 88: 1973-1998.

52. Williams RB, Barefoot JC, Califf RM, Haney TL, Saunders WB, Pryor DB et al. Prognostic importance of social and economic

resources among medically treated patients with angiographically documented coronary artery disease. JAMA 1992; 267: 520-524.

53. MacMahon S, Peto R Cutker J, Collins R, Sorlie P, Meaton J, et al. Blood pressure stroke and coronary heart disease part I. Prolonged differences in blood pressure prospective observational studies for the regression dilution bias. Lancet 1990; 335: 765-774.

54. Collins R, Mac Mahon S. Blood pressure, antihypertensive drug treatment and the risk of stroke and of coronary heart disease. Br Med Bull 1994; 50: 272-298.

55. Members of the joint national committee on detection, evaluation, treatment of high blood pressure – the fifth report of the joint national committee on detection, evaluation, treatment of high blood pressure (JNCV)

56. Cannel WB. Blood pressure as a cardiovascular risk factor: prevention and treatment. JAMA 1996; 275: 1571-1576.

57. Krwleski AS, Warram JH, Valsania P, Martin BC, Laffel LMB, Christlieb R. Evolving natural history of coronary heart disease in diabetes mellitus. Am J Med 1991; 90(suppl IIA) 56S – 61S.

58. Expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult treatment panel II). Circulation 1994; 89: 1329 – 1445.

59. Bierman E. George Lyman Duff Memorial Lecture. Atherogenesis in diabetes. Arterioscler Thromb 1992; 12:647-656.

60. Abbott RD, Donahue RP, Kannel WB, Wilson PWF. The impact of diabetes on survival following myocardial infarction in men vs women: The Framingham study. JAMA 1988; 260: 3456-3460.

61. Walden CE, Knopp RH, Walh PW, Beach KW, Strandness E Jr.Sex Differences in the effect of diabetes meelitius on lipoprotein triglyceride and cholesterol consentrrations. N Engl J Med 1984; 311:953-959

62. Reaven GM. Role of Insulin resistance in human disease. Diabetes 1998;37:1595-1601.

63. Reaven GM. Syndrome X: six years later. J Intern Med.1994;236(suppl 736);13-22.

64. Petrie JR, Ueda S, Webb DJ, Elliott HL, Connell JMC. Endothelial nitric oxide production and insulin sensitivity; A phyological link with implications for pathogenesis of cardiovascular disease. Circulation 1996;93:1331 – 1333.

65. Despres J-P, Lamarche B, Mauriege P, Cantin B, Dagenais GR, Moorjani S, et al. Hyperinsulinemia as an independent risk factor for ischemic heart disease. N Engl J Med 1996; 334:952-957.

66. Kramsch DM, Aspen AJ, Abramowiz BM, Kreimendahl T, Hood WB, Reduction of coronary atherosclerosis by moderate conditioning exercise in monkeys on an atherogenic diet. N Engl J med 1981; 305:1483 – 1489.

67. Hambrecht R, Niebauer J, Marburger C, Grunze M, Kalberer B, Hauer K et al. Various intensities of leisure time physical activity in patients with coronary artery disease : Effects on cardiorespiratory fitness and progression of coronary atherosclerotic lesions. J Am Coll Cardiol 1993: 22:468-477.

68. Haskell WL.Sedentary lifestyle as a risk factor for coronary heart disease. In: pearson TA, Criqui MH, Luepker RV, Oberman A, Winston M.eds. Primer in preventive cardiology. Dallas: American Heart Association; 1994:173-187.

69. Siscovisk DS, Weiss NS, Fletcher RH, Lasky T. The incidence of primary cardiac arrest during vigorous exercise. N Engl J Med 1984;311:874-877.

70. Pi Sunyer FX.Short term medical benefits and adverse effects of weight loss. Ann Intern Med 1993: 119(7 pt 2):722 – 726.

71. Mac Mohan SW, Wilcken DEL, Macdonald GJ. The effect of weigth reduction on left ventricular mass: A randomized controlled trial in young, overweight hypertensive patients. N Engl J Med 1986; 314-334-339.

72. Hubert HB, Fenied M, Menamara PM, Castelli WB. Obesity as an independent risk factor for cardiovascular disease: A 26 year follow

up of participants in the Framingham Heart Study. Circulation 1983: 67:968-977.

73. Manson JE, Willett WC, Stampfer MJ, Colditz GA, Hunter DJ. Body weight and mortality among women. N Engl J Med 1995; 333:677-685.

74. Jousilahti P, Tuomilehto J, Vartiaienen E, Pekkanen J, Puska P. Body weight, cardiovascular risk factors and coronary morality: 15 year follow up of middle aged men and women in eastern Finland. Circulation 1996; 93:1372-1379.

75. Larsson B. Regional Obesity as a health hazard in men: Prospective studies. Acta Med Scand Suppl 1988; 723:45-51.

76. Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. J Biol Chem 1997; 12:1258-66

77. Morel DW, Hessler JR, Chishold GM. Low density lipoprotein cytotoxicity induced by free radical peroxidation of lipid. J Lipid Res 1983; 24:1070-6.

78. Breindling KK, Alexander RW. Oxidative stress and cardiovascular disease. Circulation 1997; 96:3264-5.

79. Quinn MT, Parthasarathy S, Fong LG, Steinberg D. Oxidatively modified low density lipoproteins: a potential role in recruitment and retention of monocyte/macrophages during athergenesis. Proc Natl Sci U S A 1987; 84:2995-8.

80. Celemajer DS, Sorensen KE, Georgakopaulas D, Bull C, Thomas O, Robinson J er al. Cigaratte smoking is associated with dose related and potientially reversible improvement of endothelium dependent dilatation in young healthy adults. Circulation 1993: 88:2149 -2155.

81. Folsom AR, Wu KK, Davis CE, Conlan MG, Sorie PD, Szklo M. Population correlates of plasma fibrinogen and factor VII, Protective cardiovascular risk factors. Atherosclerosis 1991; 91:191 – 205.

82. Rival J. Riddle JM, Stein PD. Effects of chronic smoking on platelet function. Thromb Res 1987; 45:75-85.

83. Stamler J, Wentworth D, Neaton JD. Is relationship between cholesterol and risk of premature eath from coronary heart disease continous and graded? JAMA 1986; 256:2823-2828.

84. Wilson PWF. Established risk factors and coronary artery disease; The Framingham study. Am J Hypertens.1994; 7:7S-12S.

85. Peckanen J, Linn S, Heiss G, Suchindran CM, Leon A, Rifkind BM et al. Ten year mortality from cardiovascular disease in relation to cholesterol level among men with and without pre-existing cardiovascular disease. N Engl J med 1993; 328:313-318.

86. Steinberg D, Parthasarathy S, Crew TE, Khoo JC, Witztum JC. Beyond cholesterol, modification of LDL that increases the atherogenicity. N Engl J Med 1989; 320:915-924.

87. Falk E, Shah PK, Fuster V. Coronary plaque disruption. Circulation 1995; 92:657-671.

88. Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low density lipoprotein subeless patterns and risk of myocardial infarction. JAMA 260:1917-1921.

89. Hulley SB, Rosenman RH, Bawol RD, Brand RJ. Epidemiology as a guide to clinical decisions. The association between triglyceride and coronary artery disease. N Engl J Med 1980; 302:1383-1389.

90. Casetelli WP. The triglyceride issue: A view form Framingham. Am Hear J 1986; 112:432-437.

91. Zilversmit DB. Atherosclerosis: A postprandial phenomenon. Circulation 197; 60:473-485.

92. Folsom AR, Wu KK, Davis CE, Conlan MG. Population correlates of plasma fibrinogen and factor VII, putative cardiovascular risk factors. Atherosclerosis 1991; 91:191-205.

93. Gordon DJ, Probstfiedl JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, et al. High density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation 1989; 79:8 – 15.

94. Nagase H., Woessner J.F. Matrix Metalloproteinases J Priol Chem 1999; 274: 21491-21494

95. Sawaya R: Induction of tissue type plasminogen activator and 72KDa type Iv Collagenases by ionizing radiation in astrocytes. Int J Cancer 1994 56: 214-218

96. Woessner Jr: Matrix Metalloproteinase and their inhibitors in connective tissue remodeling FASEB J 1991, 5: 2145-2154

97. Moscatelli D, Rifkin DB: Membrane and matrix localization of proteinases: a common theme in tumour cell invasion and angiogenesis Biochem Biophys Acta 1988, 948: 67-85

98. Aimes RT., Quigley J.P; MMP-2 is an intertistional Collagenase: Inhibitor – free enzyme catalyzes the cleavage of collagen fibrils and soluble native type 1 collagen generating the specific ¾ and ¼ length fragments. J. Biol Chem 1995: 270: 5872-5876.

99. Pauly RR, Passanite A, Bilato C, Monticone R, Cheng L, Cron M: Migration of cultured vascular smooth muscle cells through a basement membrane barrier requires type IV collagenase activity it is inhibited by cellular differentiation. Circ Res 1994, 75: 41-54.

100. Wart H. E. V., Hansen-Birkedal H. The cysteine switch: a principal regulation of metalloproteinases activity with potential applicability to the entire MMP gene family. Proc Natl Acad Sci USA 1990, 87: 5578-5582.

101. Santaricca M., Noel A., Stoll., et al characterization of structural determinants and molecular mechanism involved in pro-stromelysin -3 by 4-aminophenyl mercuric acetate and furin type convertases. Biochem J, 1996; 315: 953-958.

102. Pei D., Majmudar G., Weiss S. J. Hydrolytic inactivation of a breast cancer cell – derived serpin by human stromelysin -3, J. Biol Chem 1994; 269: 25849-25855.

103. d'ortho MP., Stanton H., Butter M., Hembry R.M. TT1-MMP on the cell surface causes focal degradation of gelatin films. FEBS lett 1998, 421: 159-164.

104. Ellerbrock S. M., Wu Y. I., Overall C. M., Stack M. S. Functional Interplay between type 1 collagen and cell surface matrix metalloproteinase activity. J. Biol Chem 2001; 276- 24833-24842

105. Zucker S., Conner C., Dimassmo B.I., et al. Thrombin induces the activation of progelatinase A in vascular endothelial cells. J. Biol Chem 1995; 270: 23730-23738.

106. Rajagopalan S., Meng X.P., Ramasamy S., Harrison D.G., Galin Z.S. Reactiv oxygen species produced by macrophage derived forms cells regulate the activity of vascular matrix metalloproteinases invitro J. Clin. Invest 1996; 98: 2572-2579

107. Knarper V.J, Smith B., Lpes-Otin C., Morephy G. Activation of Progelatinase β (Pro MMP-9) by active collagenase-3 (MMP-13) Eur J. Bio Chem 1997, 248: 369-373.

108. V,Docherty A.J.P., Tschesche H.,(1997) Analysis of the Contribution of the hinge region of human neutrophil collagenase stability and collagenolytic activity by alanine scanning mutagenesis. FEBS Lett 405:60-64.

109. Stetler-stevenson WG. Matrix metalloproteinases in angiogenesis – a moving target for therapeutic intervention. The journal of clinical investigation. 1999; 103(9): 1237-1241.

110 Goldbach – Mansky R, Lee JM, Hoxworth JM, Smith D 2nd, Duray P, Schumacher Rh Jr, Active Synorial matrix MPP-2 is associated with radiographic erosions in patients with early synovitis, arthritis research 2000; 2(2): 145-153.

111. Zeng ZS, Cohen Am, Guillem JG. Loss of basement membrane type IV collagen is associated with increased expression of metalloproteinases 2 and 9 during human colorectal tumorigenesis 1999; 20(5): 749-755.

112 Gallis Z.S; Khatri J.J. Matrix metalloproteinase in vascular remodeling and atherogenesis: the good, the bad and the ugly. Circ Res 2002; 90: 251-262.

113 Corcoran M.L., Stetler-stevenson W.G., DeWitt D.L., Wahl L.M. Effect of Cholera toxim and pertussis taxim on prostaglandin H synthase – 2, prostaglandin E2 and matrix metalloproteinase

production by human monocytes Arch Biochem Biophys 1994; 310: 481-488.

114 Galis Z., Muszynski M., Sukhova G.K., Simon – Morrissey E., Libby P. Enhanced expression of vascular matrix metalloproteinase induced invitro by cytokines and in regions of human atherosclerotic lesions. Ann NY Acad Sci 1995; 748: 501-507.

115. Siwik D.A., Chang D.L.F., Colucci W.S. Interleukin - 1β and tumor necrosis factor - ∝ decrease collagen synthesis and increase MMP activity in cardiac fibroblasts in vitro Circ Res 2000; 86: 1259-1265.

116. Feinberg M.W., Jain M.K., Werner F., et al Transforming growth factor – β, inhibits cytokine – mediated induction of human metalloelastase in macrophages. J. Biol Chem 2000; 275: 25766-25773.

117. Uzui H., Harpf., Lice M., et al increase expression of membrane type 3 MMP in human atherosclerotic plaque. Role of activated macrophages and inflammatory cytokines. Circulation 2002; 106: 3024-3030.

118. Creemers E.E.J.M., Cleutjens J.P.M., Smits J.F.M., Daemen M.J.A.P matrix metalloproteinase inhibition after myocardial infarction: a new approach to prevent heart failure? Circ Res 2001; 89: 201-210.

119. Will H., Atkinson S., et al Membrane –type matrix 1 and 2 exhibit broad spectrum proteolytic capacities comparable to many matrix metalloproteinases .FEBS Lett 1997; 250:751-757.

120. Damas J.K., Waehre T., Yndestad A., et al stromal cell derived fctor 1-∝ in unstable angina. Circulation 2002; 106: 36-48.

121. Marx N., Schembechk U., Lazan M.A., Libby P., Plutzky J. Peroxisome proliferator-activated receptor gamma activator inhibit gene expression and migration in human vascular smooth muscle cells. Circ Res 1998; 83: 1097-1103.

122. Mtairag E.M., Oudghiri M., et al Effects of interleukin-10 on monocyte/endothelial cell adhersion and MMP-9 / TIMP-1 Secreation. Cardiovax Res 2000; 49: 882-890.

123. Davises M.J., Thomas A.C. Plaque fissuring – the cause of acute myocardial infarction, sudden ischaemic death, and crescendo angina. Br Heart J 1985; 53: 363-373.

124. Ravn H.B., Falk E. Histopathology plaque rupture cardiol clin 1999; 17:263-270.

125. Smith E.B. the influence of age and atherosclerosis on the chemistry of aortic intima. J Atherosclerosis Res 1965; 5: 241-248.

126. Shah P.K., Galis Z.S. MMP hypothesis of plaque rupture: players keep pilling up but questions remain. Circulation 2001; 104: 1878-1880.

127. Burleigh M.C., Briggs A.D., Lendon C.L. et al collagen types I and II, collagen content, GAGs and mechanical strength of human atherosclerotic plaque caps: span wise variations. Atherosclerosis 1992; 96: 71-81.

128. Moreau M., Brocheriou I., Petit L., et al Interleukin-8 mediates down regulation of tissue inhibitor – metalloproteinase -1 expression in cholesterol loaded human macro: relevance to stability of atherosclerotic plaque. Circulation 1999; 99: 420-426.

129. Noji Y., Kajinami K., Kawashiri M., et al. circulating MMPs and their inhibitor in premature coronary atherosclerosis Clin chem. Lab Med 2001; 39: 380-384.

130. Galis Z.S., Sukhova G.K., Lark M.N., Libby P. increased expression of MMPs and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J. clin invest 1994; 90: 775-778

131. Zempo N., Kenagy R.D., Au Y.P.T., et al. MMP of vascular wall cells are increased in balloon – injured rat carotid artery. J vascular surgery 1994; 20: 209-217.

132. Fernandez-Patran C., Matrinez-Cuesta M.A., Salas E., et al Differential regulation of platelet aggregation by matrix metalloproteinases 2 and 9 .Thromb Haemostat 1999; 82:1730-1735.

133. Pepper M S, Role of matrix metalloproteinases and plasminogen activator –plasmin in angiogenesis.Arterioscler Thromb Vasc Biol.2001;21:1104-1117.

134. Johnson J L,Baker Atl, Oka K,et al, Atherosclerotic plaque progression and instability by tissue inhibitor of matrix metalloproteinase 2.involv

135. Cipollene F., Prontera C., Pini B., et al. over expression of functionally coupled COX-2 and PGE synthase in symptomatic atherosclerotic plaques as a basis of PGE2 dependent plaque instability. Circulation 2001; 104: 921-927.

136. Sawicki G., Sanders E.J., Salas E., et al. localization and trans location of MMP-2 during aggregational of human platelets. Thromb haemost 1998; 80: 836-839.

137. Rademski A., Stewart M.W., Jurasz P., Randonski M. Pharmacological characteristics of solid-phase vonwillebrand factor in human platelets. Br. J. Pharmacol 2001; 134: 1013-1020.

138. Sawicki G., Salas E., Murat J., Miszta – Lane H., Radomski M.W. Release of gelatinase A during Platelet activation mediates aggregation. Nature 1997; 386: 616-619.

139. Cargili M, AH Shuler D., Ireland J., Sklar P., Ardlie K., Patil N., Lane C. R., Lim E. P., Kalayanaraman N., functional, genetic variants in human MMP-2 gene 1999 Nat. Genet. 22: 231-238.

140. Halushka M. K., Fan J.B., Bentley K., Hsie L, Shen N., Weder A. rule of SP1 in allele specific transcriptional regulation. 1999. Nat Genet. 22: 239-247.

141. Harendza S, Lovett DH, Panzer U, Lukacs Z, Kuhnl P, Stahl RA, linked common polymorphisms in the gelatenase A promoter are associated with diminished transcriptional response to estrogen and genetic fitness. J Biol Chem 2003; 278: 20490 – 20499.

142. Yuc, Zhou Y, Miao X, Xiong P, Tan W, Lin D, Functional haplotypes in the promoter of matrix metalloproteinase 2 predict risk of the occurrence and metastasis of oesophageal cancer. Cancer Res. 2004; 64: 7622 – 7628.

143. Chetty C, Rao JS, Lakka SS (2011), Matrix metalloproteinase pharmacogenomics in non-small cell lung carcinoma. Pharmacogenomics, 12: 535-46.

144. Suzuki H., Kusuyama T., Sato R., Yokota Y., Tsunoda F., Sato T. et al. 2008 Elevation of matrix metalloproteinases and interleukin-6 in the culprit coronary artery of myocardial infarction. Eur. J. Clin. Invest. 38, 166-173.

145. Park H. Y., Kwon H. M., Lim H. J., Hong B. K., Lee J. Y., Park B. E. et al. 2001 Potential role of leptin in angiogenesis: leptin induces endothelial cell proliferation and expression of matrix metalloproteinases in vivo and in vitro. Exp. Mol. Med. 33, 95-102.

146. Choi J. Y., Bae J. S., Kim Y. A., et al. clinical significance of MMP-2, MMP-9, and HIF-1 ∝ expression in thyroid micropapillary cancer. J. Korean Surg soc. 2010; 78(3): 157-164.

147. Libra M, Scalisi A, Vella N. uterine cervical Carcinoma : role of matrix metalloproteinase (review). Int J On col. 2009; 34 (4): 897-903.

148. Chakrabarti S, Patel K. D., Matrix metalloproteinase 2 (MMP-2) and MMP-9 in pulmonary pathology. Exp lung Res. 2005; 31: 599-621.

149. Mia O. X., Yu C., Tan W., Xiong P, Liang G, Lu W, Lin D. A functional polymorphism in matrix metalloproteinase 2 gene promoter is associated with risk of development of but not metastasis of gastric cardia adenocarcinoma. Can Res. 2003; 63: 3987-3990.

150. Hegab AE, Sakamoto T, Saitoh W, Association analysis of tissue inhibitor of metalloproteinase 2 gene polymorphism with COPD in Egyptians. Respir Med. 2005; 99: 107-110.

151. Xu E, Lai M, Lu B, Xing X, Huang Q, XiaX. A single nucleotide polymorphism in the matrix metalloproteinase 2 promoter is associated with colorectal cancer. Bio Chem Bio Phys Res. Commun 2004; 324: 999-1003.

152. Lin SC, Lo SS, Liu CJ, Chung MY, Huang JN, Chang KW. Functional genotype in matrix metalloproteinase 2 promoter is a risk factor for oral carcinogenesis J oral pathol Med. 2004; 33: 405-409.

153. Pacheco M. M., Mourao M., Mantovani E. B., Expression of gelatinase A and B in breast carcinomas: clinico-pathological correlations. Clin. Exp. Metastasis, 16: 577-585, 1998.

154. Boag A. H., Young I. D., Increased expression of 72KD type IV collagenase in prostatic adenocarcinoma. Demonstration by Immunochemistry and in situ hybridization. Am J. Pathol., 144: 585-591, 1994.

155. Dumar V., Kanitakis J, Faure M, Expression of basement membrane antigene and matrix metalloproteinase 2 and 9 in cutaneous basal and sq uamous cell carcinomas. Anticancer Res, 19: 2929-2938, 1999.

156. Davis V, Persidskaia R, Baca-Ragen L, et al matrix metalloproteinase 2 production and its binding to the matrix are increased in abdominal aortic aneuyrsms. Arterioscler thromb Vasc Biol. 1998; 18: 1625-1633.

157. Sivula A, Lundin J, Haglund C, Association of COX 2 and MMP-2 expression in human breast cancer. Breast cancer Res. 2005; 89(3):215-20.

158. Eva Senn, Thomas F, Michael G. Single nucleotide polymorphisms of MMP-2 gene in stroke subtypes. Cerebro vasc Dis 2008; 26: 113-119.

159. Deb S, Zhang, J. W., Gottschall P.E.J. Activated isoforms of MMP-2 are induced in U87 human gliomo cells in response to beta amyloid peptide. 1999; 5: 252-256.

160. Iki, K., Kido, A., Tsunodo, T., Expression of MMP-2, and activation of pro MMP-2 in pancreatic duct adenocarcinoma carcinogenesis (1999).

161. Ageel, A. A., Sheth, K. V., Eid, W. A., Meyer, B. F., mutation of MMP-2 causes a MCOsteolysis and arthritis syndrome. Genet. (2001)

162. Lander ES, Schor NJ. Genetic dissection of complex traits. Science 1994; 265: 2037-2048.

163. Hisashi Kai MD,PhD. Maniko kai PhD,Yukihikoseki MD, Peripheral blood levels of MMP-2 elevated in patients with MI, Journal of American College of Cardiology Vol 32,Issue 2,1998.

ANNEXURES

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI -3

Telephone No: 04425305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. B. Sudhapresanna
PG in MD Biochemistry
Madras Medical College, Chennai -3.

Dear Dr. B. Sudhapresanna

The Institutional Ethics Committee of Madras Medical College reviewed and discussed your application for approval of the proposal entitled "Association of matrix metalloproteinase -2 gene promoter polymorphism with myocardial infarction" No. 02072011.

The following members of Ethics Committee were present in the meeting held on 21.07.2011 conducted at Madras Medical College, Chennai -3.

1.	Prof. S.K. Rajan, MD	-- Chairperson
2.	Prof. V. Kanagasabai, MD Dean, Madras Medical College, Chennai-3,	-- Deputy Chairman
3.	Prof. A. Sundaram, MD Vice Principal , Madras Medical College, Chennai -3	-- Member Secretary
4.	Prof R. Sathianathan , MD	-- Member
5.	Prof R. Nandhini, MD Director, Institute of Pharmacology, MMC, Ch-3	-- Member
6.	Prof. Geetha Subramanian MD. DM Prof & Head , Dept. of Cardiology, MMC, Ch-3	-- Member
7.	Prof. Pregna B. Dolia, MD Director , Institute of Biochemistry, MMC, Ch-3	-- Member
8.	Prof. C. Rajendiran, MD Director , Institute of Internal Medicine, MMC, Ch-3	-- Member
9.	Thiru. A. Ulaganathan Administrative Officer, MMC, Chennai -3	-- Layperson
10.	Thiru. S. Govindasamy . BA.BL	-- Lawyer
11.	Tmt. Arnold Soulina MA	-- Social Scientist

We approve the proposal to be conducted in its presented form

Sd /. Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information / informed consent and asks to be provided a copy of the final report

Member Secretary, Ethics Committee

PATIENT CONSENT FORM

Title of the study **: "ASSOCIATION OF MATRIX METALLOPROTEINASE-2 GENE PROMOTER POLYMORPHISM WITH MYOCARDIAL INFARCTION. "**

Name : Date :

Age : OP No :

Sex : Project Patient No :

The details of the study have been provided to me in writing and explained to me in my own language.

I confirm that I have understood the above study and had the opportunity to ask questions.

I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected.

I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).

I have been given an information sheet giving details of the study.

I fully consent to participate in the above study.

Signature

No.	

ASSOCIATION OF MATRIX METALLO PROTEINASE-2 GENE PROMOTER POLYMORPHISM WITH MYOCARDIAL INFARCTION

PROFORMA

Name: Age/Sex: IP /OP No:

Address: Phone No:

Ward

Diagnosis:

Presenting complaints: Duration:

Past history:

Chest pain

Hypertension

Diabetes

Personal History:

Smoking

Alcoholism

Tobacco chewing

Menstrual history

Diet history

Family history

<u>Examination</u> :

Vital data :

Heart Rate :

Blood pressure (mean) :

Height :

Weight :

Systemic examination :

CVS

RS

ABDOMEN

CNS

Impression

<u>Investigations:</u>

Fasting lipid profile

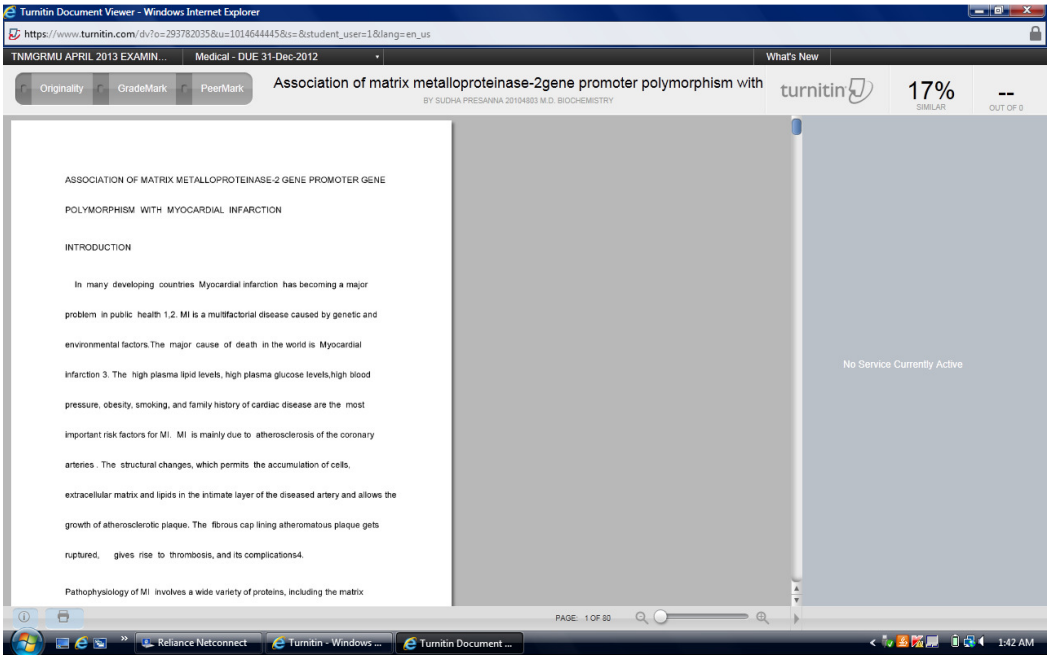
Plasma MMP-2 estimation by ELISA

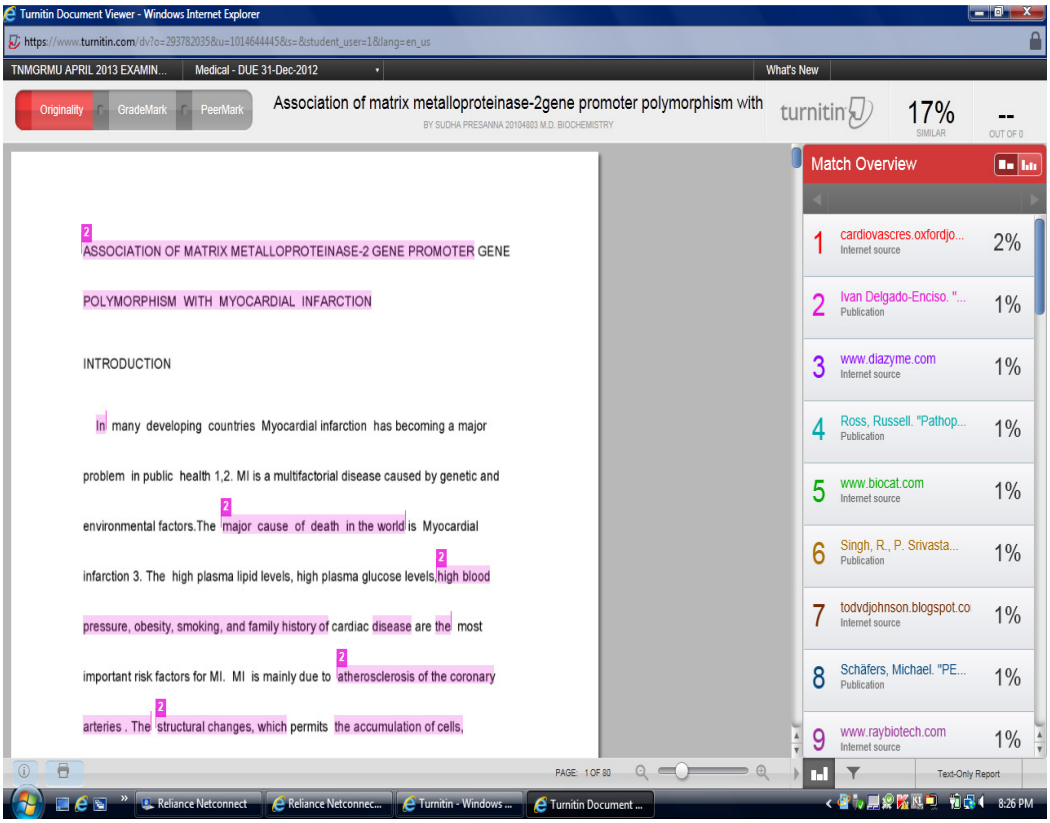
Genotyping by polymerase chain reaction and restriction fragment length polymorphism.

Coronary Angiography

ECG.

SIMILARITY CHECK 17% SIMILARITY




Turnitin Document Viewer - Windows Internet Explorer
https://www.turnitin.com/dv?o=293782035&u=1014644445&s=&student_user=1&lang=en_us
TNMGRMU APRIL 2013 EXAMIN...
Medical - DUE 31-Dec-2012
What's New
Originality
GradeMark
PeerMark
Association of matrix metalloproteinase-2gene promoter polymorphism with
BY SUDHA PRESANNA 20104803 M.D. BIOCHEMISTRY
turnitin
17%
SIMILAR
--
OUT OF 0
ASSOCIATION OF MATRIX METALLOPROTEINASE-2 GENE PROMOTER GENE
POLYMORPHISM WITH MYOCARDIAL INFARCTION
INTRODUCTION
In many developing countries Myocardial infarction has becoming a major
problem in public health 1,2. MI is a multifactorial disease caused by genetic and
environmental factors.The major cause of death in the world is Myocardial
infarction 3. The high plasma lipid levels, high plasma glucose levels,high blood
pressure, obesity, smoking, and family history of cardiac disease are the most
important risk factors for MI. MI is mainly due to atherosclerosis of the coronary
arteries . The structural changes, which permits the accumulation of cells,
PAGE: 1 OF 80
Match Overview
1 cardiovascres.oxfordjo... Internet source 2%
2 Ivan Delgado-Enciso. "... Publication 1%
3 www.diazyme.com Internet source 1%
4 Ross, Russell. "Pathop... Publication 1%
5 www.biocat.com Internet source 1%
6 Singh, R., P. Srivasta... Publication 1%
7 todvdjohnson.blogspot.co Internet source 1%
8 Schäfers, Michael. "PE... Publication 1%
9 www.raybiotech.com Internet source 1%
Text-Only Report
Reliance Netconnect
Reliance Netconnec...
Turnitin - Windows ...
Turnitin Document ...
8:26 PM